Final Synopsis

"Bioactive Peptides from Mangrove Soil Actinomycetes and Characterization for Antimicrobial and Anticancer activity"

Final synopsis submitted to

MANGALORE UNIVERSITY

For the award of the degree of

DOCTOR OF PHILOSOPHY

In

MICROBIOLOGY

Submitted by

Mr. Y Karthik

(Registration No. MU/EXB/Ph.D/CR.22/Micro./2020-21/E.13)

Research Supervisor

Dr. I. K. Manjula M.Sc., PhD.

Department of Studies and Research in Microbiology
Mangalore University, Jnana Kaveri, Chikka Aluvara,
Kodagu (District), Karnataka, India- 571232

August - 2022

CONTENTS

Serial No	Index	Page No.
1.	Introduction	1-5
2.	Review of Literature	6-14
3.	Results and Discussion	15-53
4.	Major findings and Conclusion	54-56
5.	Appendices	56-59
6.	References	54-61

Chapter - 1 Introduction

Microorganisms constitute approximately 60 % of the earth's living matter and two to three billion species share the earth, highly influencing the lives of all biotic factors. The microbial world, a treasure in itself, if utilized judiciously, can contribute to the sustainable development. They are significant in the subsistence of an ecological balance on the globe (Kuhad, 2012). As the envoy of contagious disease, they become source for unlimited human afflictions, yet their advantageous roles are multitudinous ranging from the biogeochemical cycles through to the production of food, beverages, cosmetics, leather industries and pharmaceuticals. Microorganisms have been recognized as rich sources for extraction of biologically active molecules that find wide range in exploitation in medicine, agriculture and industry (Monciardini et al., 2014).

They are considered as potential candidates for solving more complex problems in the field of science and technology because of their unique and inconsistent nature and bio-synthetic capabilities. In the past 80 years, they have helped enhance medical technology, food and dairy technology (processing, safety, and quality), genetic engineering, environmental protection, agricultural biotechnology, and the agricultural and municipal waste treatment industries. (Higa, 1994; Raaijmakers et al., 2009).

Major groups of microorganisms include bacteria, fungi and specific bacterial taxa namely *Actinomyces*. Among microbes, the *Actinomyces* signify the most economically and biologically significant bacteria as they produce diverse bioactive compounds, namely antibiotics, antitumor and immunosuppressant agents, inhibitors, enzymes and plant growth promoting substances. Undoubtedly, *Actinomyces* symbolize the major opportunity to achieve further research advancements that could be applicable in the field of medicine, agriculture and other industries

(Chaudhary et al., 2013). *Actinomyces* are extensively distributed group of microorganisms, secreting a wide range of natural products in their surroundings, which contributes protection against pathogens. They also play important role in decomposition and humus formation for soil enrichment and involved in reducing soil erosion. They are of special interest, in view of the fact that the bioactive products from *Actinomyces* have not only revealed the significance in biotechnological and pharmaceutical applications but have also enlarged our understanding of the diversity of microbiota. *Actinomyces* are aerobic Gram positive bacteria that form branching usually non-fragmenting hyphae and asexual spores. They represent one of the dominant phyla in the domain bacteria confining to the five classes nineteen orders and fifty families according to the Bergey's manual of systematic bacteriology (Goodfellow et al., 2012; Zucchi et al., 2012).

These mangroves are having distinct features such as variation in geographic location, high pH, polythermal ensemble, high salinity, high tidal waves, moisture and stringent nutrient condition in different regions of mangroves. Therefore, they cherish diverse and unique *Actinomyces* genera. Mangroves are capable of absorbing up to four times as much carbon dioxide as upland terrestrial forests, determined by area (Donato et al., 2012, 2011). The astonishing characteristics of the mangrove ecosystem result in a wide range of benefits for us in the form of products and services. These are essential for maintaining human well-being since they provide things like food, shelter, and means of subsistence. The vulnerability of local residents is also decreased by mangroves, reducing property damage. Mangroves are being destroyed, lost, or inadequately restored at a startling rate despite the inestimable advantages they provide, which are estimated to be worth US$33-57,000 per hectare (Duke et al., 2014). Although it has since stabilised, between 1980 and 2000, mangroves lost about 35% of their area, which is 3-5 times more than the total loss of terrestrial forests (Duraiappah et al., 2005). Weak institutional structures,

policies, and management systems further intensify threats. Furthermore, the populations that depend on the mangroves frequently overuse the diminishing resources due to concerns with poverty and fairness.

To date, classification under the order *Actinomycetales*, eight suborders, 11 families and 24 genera including three new genera have been reported from the mangrove sediments (Gunatilaka, 2006; Tian et al., 2014; Xu et al., 2014). With the above background, a study was initiated with the objective to estimate the population of *Actinomyces* in Mangrove soils.

Actinomyces are physiologically active group renowned for their secondary metabolites that are explored with innumerable research areas and extensive applications. While it may be true that a few *Actinomyces* are pathogenic for humans, animals or plants such as *Streptomyces somaliensis* which causes actinomycetoma (Kirby et al., 2012), *Actinomyces bovis* causes lumpy-jaw disease of cattle (Ahad, 2020), *Streptomyces scabies* causes common scab in potatoes and sugar beets (Ismail et al., 2020). The secondary metabolites of *Actinomyces* origin find wide range of applications as a potential source of novel compounds with antiviral, antibacterial, anti-parasitic, antifungal, anti-tumour, immuno-suppressants, biocontrol agents, antihelminth, agro active compounds, growth regulators, enzymes, enzyme inhibitors and other properties (Janiszewska et al., 2012; Kalyani, 2017; Mahmoudi et al., 2016; Solecka et al., 2012). As yet, soil *Actinomyces* remain the best source for the isolation of bioactive metabolites with antibiotic, enzymatic and plant growth promoting or inhibiting activities. A report that about 45% of all bioactive secondary metabolites of microbial origin are obtained from the *Actinomyces* and approximately 75% is produced by *Streptomyces sp.* (Abdillah et al., 2015; Kalyani, 2017).

Chapter –II Review of Literature

The proteins secretions by *Streptomyces* are produced as pre-proteins comprising of mature protein prolongs with N-terminal regulating peptide which will be hydrolyzed off during translocation process across the membrane. Even though primary sequences are rarely conserved amidst regulatory peptides, they all possess typical tripartite confirmations: an alkaline amino-terminus, a central apolar centric and a carboxy-terminal region comprising the regulatory enzymes recognition site. The present available knowledge of *Streptomyces* leader sequences and the impact of introduced mutations on transcription, translation and secretion of homologous and heterologous proteins are extensive (Lammertyn and Anne, 1998).

The isolation of lasso peptides is ribosomally produced and post-translationally modified peptides produced by bacteria. They are characterized by an unusual lariat-knot structure. The genome sequence shown a wide diversity of lasso peptides encrypted in bacterial genome library, An orthogonal regulatory system for heterologous synthesis of bacterial lasso peptides in *Streptomyces* hosts based on a newly-identified regulatory circuit from *Actinoalloteichus fjordicus* was achieved. Varying the *Streptomyces* expression hosts with a miniscule set of culture conditions, out of six lasso peptide gene clusters, mainly originating from marine *Actinobacteria*, three new lasso peptides were successfully produced and elucidated by tandem MS. The newly developed expression system thus sets the stage to uncover and bioengineer the chemo-diversity of *Actinobacterial* lasso peptides. Moreover this data provided some considerations for future bio-prospecting efforts for such peptides isolated from *Streptomyces* (Mevaere et al., 2018).

The isolation, detailed applications and characterization for the latest non-ribosomally synthesized antibiotic 5812-A/C was derived from metabolites of Streptomyces roseoflavus. According to its chemical confirmations, the studied 5812-A/C basically composed of a cyclic

peptide part covalently attached with an arabinose amino acid. N-terminal residual sequencing of the native peptide has distinguished its partial structure of Leu-Asp-Gly-Ser-Gly and comprising of a Tyr amino acid that is expected to have a two-component peptide nature for the molecule studied. However, the structural conformity of the antibiotic complex derived from S. roseoflavus INA-Ac-5812 is still ongoing. The mechanism of action of 5812-A/C was assessed in comparison with its most related analog, the lipo-peptide antibiotic daptomycin, given the presence in both antimicrobials of an L-kynurenine amino acid residue. The inhibitory activity of 5812-A/C against Gram-positive bacteria including methicillin-resistant strain of Staphylococcus aureus was similar to daptomycin. The mechanism of action of 5812-A/C was associated with the disruption of membrane integrity, which differs in comparison with daptomycin and is most similar to the antimicrobial membrane-disturbing peptides. However, 5812-A/C demonstrated a calcium-dependent mode of action. In addition, unlike daptomycin, 5812-A/C was able to penetrate mature biofilms and inhibit the metabolic activity of embedded S. aureus cells. At the same time, 5812-A/C has no hemolytic activity toward erythrocyte, but possessed weak cytotoxic activity represented by heterochromatin condensation in human buccal epithelium cells. The biological properties of the peptide 5812-A/C suggest its classification as a calcium-dependent antibiotic effective against a wide spectrum of Gram-positive pathogenic bacteria (Vasilchenko et al., 2020).

Another study also described the elucidation of a lasso peptide albusnodin possessing post-translational addition with an acetyl group, first example of a lasso peptide with this modification. Using heterologous expression, further showed that the acetyl-transferase adjoining with the albusnodin gene cluster is required for the biosynthesis of this lasso peptide.

This type of lasso peptide is widespread in *Actinobacteria* with 44 examples found in currently sequenced genomes (Zong et al., 2018).

The bioactive compound avermectin a macro cyclic polyketide produced from *Streptomyces avermitilis.* This bioactive compound is well known for anthelmintic in medical, veterinary and agricultural fields. The genes aveR is present on the left-hand extremity of the avermectin biosynthetic gene cluster and the deduced gene product showed similarity to the LAL-family (large ATP binding regulators of the LuxR family) transcriptional regulators. This aveR posses an controlling gene for the production of commercially available avermectin (Kitani et al., 2009).

Other study from Ivana Charousova explored 86 isolates from aridic soil from Karoo of South Africa. Out of which 35 distinctive cultures were chosen, 10 isolates showed efficient antimicrobial and antifungal activity. The whole cell protein isolation resulted in the identification of a bioactive actinomycin peptide from isolate *Streptomyces antibioticus.* This actinomycin was characterized by using HPLC, MALDI-TOF/MS and LCMS analysis led to identification of active peak at 442nm and confirmed using ESI-HRMS (Charousova et al., 2019).

The assemblage of proteins into amyloid fibrils offered an alternate structural configuration to the natively folded state of most polypeptides for structure function stablity. The amyloid fibrils represented the natural functional state of some proteins, such as the chaplins from the soil-dwelling bacterium *Streptomyces coelicolor*, even though it is commonly associated with disease, which covers the aerial mycelium and spores contributing for hydrophobicity. A biophysical characterization of the five short chaplin peptides ChpD-H assemblage in solution to form fibrils was probed for the mechanism of these peptides. Each of the five chaplin peptides produced or isolated from the cell wall is individually surface-active and capable of forming

fibrils under a range of solution conditions *in vitro*. These fibrils contain a highly similar cross-β core structure and a secondary structure that resembles fibrils formed *in vivo* on the spore and mycelium surface. Also showed restoration of aerial hyphae growth in a chaplin mutant strain (Sawyer et al., 2011).

The production of bioactive compound avermectin from *Streptomyces avermitilis.* Here, 2-dimensional electrophoresis (DE) was first attempted to analyze the proteome profile of *Streptomyces avermitilis* during batch fermentations in complex medium. In this process, the first key point was to extract the protein of the organism from the complex using tri-chloro acetate acetone to precipitate, re-solubilized and characterised avermectin in 8 M urea (Yin et al., 2008).

The *Actinomyces sp.* viz., *Actinomadura sp. Strepomyces cyaneus* and *Thermomospora mesophila* were checked for their ability to solubilize ligno-cellulose complex. Each organism was incubated in either glucose or ball milled straw for 17 d at 30 ± 2 ^{o}C along with ^{14}C labeled ligno-cellulose material. The produced extracellular proteins were characterized by using SDS-PAGE and gel filtration of the extracellular proteins, using superose 12. The isolate *Strepomyces cyaneus* on ball milled straw was able to solubilize lignin from lignocellulose (Clark Mason et al., 1988).

The *Streptomyces lividans* is one isolate lacking restriction system compared to other *Streptomyces spp.* Hence it is widely used for protein production and implicated in therapeutic applications. This isolate also possessed low endogenous extracellular proteolytic activity as a result it was quite familiarly easy to perform genetic manipulation for production of recombinant proteins (Binnie et al., 1997; Johansson E et al., 2009). The *Streptomyces parvulus* was isolated from Bay of Bengal marine soil samples, Vishakhapatnam, Andrapradesh. This species could able to produce maximum number of protein encapsulated silver nanoparticles on just 24 hr of

incubation. These proteins were shown significant activity against different Gram-negative pathogenic bacteria (Prakasham et al., 2014).

The soil samples from fish cultivating reservoirs in Coimbatore district, Tamilnadu offered isolation of twenty distinctive *Actinomyces* strains belonging to genus *Streptomyces sps.* The isolate BR-13 produced crude protein extract by acetone precipitation method, the resulted protein was about 49 and 65 kDa and showed potential antagonistic activity against clinically isolated well known Gram-negative bacteria *Salmonella typhimurium* (Poffe et al., 2014). The two recent cyclic peptides dehydroxy and desmethylenyl along with nocardamine were isolated from the broth culture of an *Actinomyces* belonging to *Streptomyces* genus isolated from an unexplored marine sponge. The novel compounds showed less inhibition against the enzyme sortase B (Hyi-Seung Lee et al., 2005).

Ohmyungamycins A and B (1 and 2), are novel cyclic peptides characterized from marine bacterial cultures belonging to the genus *Streptomyces* were collected from sand beach on Jeju, volcanic island, the Republic of Korea. Based on different techniques and advancements such as NMR, UV, IR spectroscopy and MS data the compounds are illustrated as cyclic desipeptides showing unusual amino acid residues includes N-methyl-4-methoxytrytophan, N,N-dimethylvaline and β-hydroxyphenylalanine. The configurations of α-carbons of the amino acid residue were elucidated using Marfey's method. The additional stereogenic centers at the β-carbons like threonine, N-methylthreonine and β-hydroxyphenyalanine amino acids were allotted by GITC (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocynate) derivatization and the modified Mosher's method utilizing PGME (phenyl-glycine methyl ester) joined with upon chromatographic studies N,N-dimethylvaline. The ohmyungsamycins A and B was demonstrated for potential inhibition against diverse cancer and pathogenic bacteria cultures (Um et al., 2013).

The pargamicin A, novel cyclic hexapeptide antibiotic was cultured from the culture broth of an *Actinomyces* strain. The producing organism, designated ML1-hF4, was marked as a member of the genus *Amycolatopsis*. This cyclic hexapeptide containing piperazic acid showed potent antibacterial activity against *S. aureus* strains including MRSA and *Enterococcus faecium* strains (Igarashi et al., 2008). Another study on new cytotoxic cyclic peptide YM-216391 isolated from the cultured mycelium of *Streptomyces nobilis*. The planar structure of YM-216391 was assigned on the basis of 1D and 2D NMR spectroscopic techniques. The absolute configuration of the amino acid residues in YM-216391 was evaluated by Marfey's and chiral HPLC analysis (Sohda et al., 2005).

The strain *Streptomyces cacaoi* CA-170360 produced the cyclic penta-peptides pentaminomycins A-E and BE-18257 A-C, two families of cyclo-peptides synthesized by two NRPS encoded in tandem within the same biosynthetic gene cluster. The cloned heterologous expression of this biosynthetic gene clusters elucidated that each of the NRPS present in the cluster is involved in the biosynthesis of each group of cyclopeptides. In addition, reports on the involvement of a stand-alone enzyme belonging to the penicillin binding protein family in the release and macro-cyclization of the peptides for many genetic studies (Roman-Hurtado et al., 2020).

The *Actinomyces* isolates of *Streptomyces champavatii* were isolated from marine soil sediments of the Gotland Deep (Baltic Sea), Urania Basin (Eastern Mediterranean), from the Kiel Bight (Baltic Sea). The *Actinomyces* isolates produced many oligopeptidic secondary metabolites, including the new octapeptide champacyclin. Studies on sequence and configuration of stereo-centers were obtained by a combination of multi stage mass spectrometry studies, amino acid analysis, partial hydrolysis and subsequent enantiomer analytics by GC-PCI/EI-MS analysis.

Proof on the head-to-tail cyclization was accomplished by solid phase peptide synthesis (SPPS) compared to an alternatively side chain cyclized derivative. Champacyclin is likely synthesized by a NRPS, because of its high content of (d)-amino acids. The compound showed antimicrobial activity against the phyto-pathogen *Erwinia amylovora* causing the fire blight disease of certain plants (Pesic et al., 2013).

Another study described three new cyclic heptapeptides, cyclomarins A-C (1-3), were isolated from extracts of a cultured marine bacterium collected in the vicinity of San Diego, CA. The major metabolite, cyclomarin A (1), contains three common and four unusual amino acids. The structure of the new metabolites were determined using 1D and 2D NMR method, and the stereochemistry was determined from an X-ray crystal structure of the diacetate derivatives. Cyclomarin A(1) displayed significant anti-inflammatory activity in both *in vivo* and *in vitro* assays (Renner et al., 1999).

Recent identification and genetic studies of the biosynthetic gene cluster responsible for synthesis of the ribosomally synthesized and post-translationally differentiated peptide cypemycin revealed a new class of peptide natural products, the linaridins. Another peptide grisemycin, a linaridin synthesized by a gene cluster in *Streptomyces griseus*. Mass spectrometric analysis revealed that grisemycin possessed at least three of the modifications found in cypemycin, as well as an analogous leader peptidase cleavage site. Expression of putative grisemycin biosynthetic genes in a *Streptomyces coelicolor* derivative, combined with deletion of the gene encoding the grisemycin precursor peptide, confirmed the identity of the grisemycin gene cluster. Both grisemycin and cypemycin depend on the transcriptional activator AdpA for wild-type levels of production (Claesen and Bibb, 2011).

Cyclic peptides are commonly used as quorum-sensing auto-inducers in Gram-positive *Firmicutes* bacteria. Well-studied illustrations of such molecules are thiolactone and lactone, used to induce the expression of a series of virulence genes in the *agr* system of *Staphylococcus aureus* and the *fsr* system of *Enterococcus faecalis* consequently. Three cyclodepsipeptides WS9326A, WS9326B and cochinmicin II/III were identified as a result of screening actinomycetes culture extracts for activity against the *agr*/*fsr* system. These molecules are well known as receptor antagonists, the first two for tachykinin and the last one for endothelin. WS9326A also inhibited the transcription of *pfoA* regulated by the VirSR two-component system in *Clostridium perfringens*. Receptor-binding assays using a fluorescence-labeled autoinducer (FITC-GBAP) showed that WS9326A and WS9326B act as receptor antagonists in this system. In addition, an *ex vivo* assay showed that WS9326B substantially attenuated the toxicity of *S. aureus* in human corneal epithelial cells. These results suggested that these three natural cyclodepsipeptides have therapeutic potential for specifically targeting the cyclic peptide-mediated quorum sensing of Gram-positive pathogens (Desouky et al., 2015).

Latest seven halogenated peptides termed svetamycins A–G (**1**–**7**) have been isolated from laboratory cultures of a *Streptomyces sp.* Svetamycins A-D, F, and G are cyclic depsipeptides, the svetamycin E is a linear analogue of svetamycin C. Svetamycins are distinguished by the existence of a hydroxyl acetic acid and five amino acids residue including a rare 4,5-dihydroxy-2,3,4,5-tetrahydropyridazine-3-carboxylic acid, a γ-halogenated piperazic acid, and a unique δ-methylated piperazic acid in svetamycins B–C, E, and G. Furthermore, isotope-labeled substrate feeding appraisal illustrated ornithine as the precursor of piperazic acid and that methylation at the δ position of the piperazyl scaffold is *S*-adenosyl-L-methionine (SAM)-dependent.

Svetamycin G, the most potent antimicrobial of this suite of compounds, inhibited the growth of *Mycobacterium smegmatis* with an MIC_{80} value of 2 μg/ml (Dardic et al., 2017).

Objectives:

I. Isolation of mangrove-soil actinomycetes and their molecular characterization.

II. Extraction and selection of mangrove actinomycetes producing bioactive peptides with antimicrobial and anticancer activity.

III. Purification and structural characterization of bioactive peptides and their mechanism of action towards antimicrobial and anticancer activity.

Chapter-3 Results and Discussion

4.1 Isolation of mangrove soil *Actinomyces*

The soil sample was randomly collected in four different sites of mangroves habitat on 24thJune of 2018, in Mangalore region, Dakshina Kannada, Karnataka, India. The four soil sample sites and their premises such as Jeppinamogaru (JPMU) 12°50'31.4"N 74°51'36.4"E. Adam Kudru (AMKU) 12°50'02.3"N 74°51'46.7"Ein Kallapu (KLPU) 12°50'07.3"N 74°51'31.2"E and Ashok Nagar, Tannirbavi (ANTB) 12°54'16.5"N 74°49'09.4"E. The soil samples were collected from a depth of 8 inches carried using sterile sampling zip-lock bags (Tarsons Pvt. Ltd).

The unique environmental conditions and diversified flora and fauna in the mangrove habitat in the coastal region of Mangalore offered a natural resource for isolation and identification of unstudied *Actinomyces*. Therefore the mangrove soil samples collected from four different geographical sites**.** Each collected mangrove soil exhibited distinctive soil texture and color, sandy texture and black in color at Ashok nagar, Tannir bavi. Adam kudru soil sample was red colored loamy soil. Black and brown colored with powdery texture soils were from Jeppinamogaru and Kallapu respectively. Physical parameters such as temperature of 21^{o}C ±2^{o}C and pH 7.2 were recorded. For isolation of mangrove *Actinomyces*, the soil samples were treated with 0.2% $CaCO_3$ and preheated at 60 ^{o}C for 2 hr for reducing fungal and bacterial loads. Isolation procedure was standardized using different protocols and employing soil pretreatment by using chemical and heat treatment at different temperatures. Studies have reported successful isolation of *Actinomyces* after preheat treatment at 72 ^{o}C for 2hr (Doshi et al., 2010). 65 ^{o}C for 10min using rotary shaker (Balasubramanian et al., 2021), 50 ^{o}C for 30min (Myla et al., 2016).

Incubating the soil samples at 60°C for 1 hr in oven along with 0.1 % $CaCO_3$ were reported (Krishanti, 2018).In another study, isolation procedure for *Actinomyces* from soil samples were heated at 70°C for 15 min followed by soil dilution procedure increased isolation from 3 folds to 7 folds (Myla et al., 2016; Ramachandran and Kuppusamy, 2016). Subsequently, after serial dilution, 0.1 ml from the seven-fold dilution sample was pour plated on different media such as Kustar's agar (KA), Isolation agar (IA), Yeast malt extract agar (YMA),Yeast extract agar (YEA)(Rajeswari et al., 2015)and Starch casein nitrate agar (SCNA)incorporated with amphicilin and nystatinas shown in **Figure 14-18.** A significant number of distinctive *Actinomyces* colonies were grown on SCNA and YEA media. Similar studies discussed that SCNA media supplemented with different antibiotics for isolation of *Actinomyces* with Ampicillin of 0.0020 % and Fluconazol of 0.0025 %and with differential concentrations inhibited the growth of bacterial and fungal contaminants respectively(El Karkouri et al., 2019; Sapkota et al., 2020; Shrestha et al., 2021; Williams and Davies, 1965). Isolation agar medium supplemented with cyclohexamide 0.0050 % and nystatin 0.0050 % for *Actinomyces* isolation was studied by Chaudary(Hotam S. Chaudhary et al., 2013).

In our study we compared the growth *Actinomyces* colonies on five distinct media, the SCNA and YEA media with pH 7.2 supported the optimal growth of *Actinomyces* colonies. Collectively from the two selected media 87 *Actinomyces* isolates out of which forty one cultures from SCNA media and 46 *Actinomyces* colonies from YEA media were picked and sub-cultured. The distinctive *Actinomyces* colonies were studied for their varied morphological characteristics shown in **Table 4.1** for YEA *Actinomyces* isolates and **Table 4.2** detailed for SCNA *Actinomyces* isolates. Therefore, this study was able to explore 87 distinct Actinomyces isolates from four different mangrove soils

of Mangalore region. Further these 87 *Actinomyces* isolates were stored in 50 % glycerol for longtime storage. Each isolates were characterized by microscopic and macroscopic structural analysis for pigmentation, shape, texture, Gram stain and spore structure analysis.

Table 4.1 Cultural characteristics of mangrove soil *Actinomyces* grown on YEA media.

***Actinomyces* Isolate**	**Texture**	**Pigmentation**	**Aerial mycelia color**	**Substratum color**	**Gram Staining**	**Elevation**	**Shape**
Y1	Powdery	+ve	Grey	Brown	+ve	Elevated	Circular
Y2	Slimy	+ve	Ash Black	Black	+ve	Flat	Circular
Y3	Powdery	+ve	White	Red	+ve	Elevated	Irregular
Y4	Powdery	+ve	White	Black	+ve	Flat	Circular
Y5	Slimy	+ve	Black ash	Brown	+ve	Elevated	Irregular
Y6	Slimy	+ve	Ash	Brown	+ve	Elevated	Circular
Y7	Powdery	+ve	Pink	Pink	+ve	Elevated	Irregular
Y8	Slimy	+ve	White	Orange	+ve	Elevated	Circular
Y9	Slimy	+ve	White	Pink	+ve	Elevated	Circular
Y10	Powdery	+ve	Light red	Brown	+ve	Flat	Circular
Y11	Slimy	+ve	Grey	Brown	+ve	Elevated	Irregular
Y12	Slimy	+ve	White	Pink	+ve	Elevated	Circular
Y13	Powdery	+ve	Light yellow	Orange	+ve	Flat	Circular
Y14	Powdery	+ve	White	Pink	+ve	Elevated	Circular
Y15	Slimy	+ve	Pinkish	Light brown	+ve	Elevated	Irregular
Y16	Powdery	+ve	Grey	Orange	+ve	Elevated	Irregular
Y17	Slimy	+ve	Grey	Orange	+ve	Elevated	Circular
Y18	Powdery	+ve	Black	Orange	+ve	Elevated	Circular
Y19	Slimy	+ve	Orange	Orange	+ve	Elevated	Circular
Y20	Powdery	+ve	Grey	Red	+ve	Elevated	Circular
Y21	Powdery	+ve	Black	Brown	+ve	Elevated	Circular
Y22	Powdery	+ve	Grey	Brown	+ve	Flat	Irregular
Y23	Powdery	+ve	Black	Brown	+ve	Flat	Circular
Y24	Powdery	+ve	Black	Brown	+ve	Elevated	Irregular
Y25	Slimy	+ve	Orange	Orange	+ve	Elevated	Circular
Y26	Slimy	+ve	Grey	Orange	+ve	Elevated	Circular

Y27	Powdery	+ve	White	Black	+ve	Elevated	Circular
Y28	Slimy	+ve	Grey black	Colorless	+ve	Elevated	Circular
Y29	Slimy	+ve	Grey creamish	Colorless	+ve	Elevated	Circular
Y30	Powdery	+ve	Black	Orange	+ve	Elevated	Irregular
Y31	Powdery	+ve	Black	Brown	+ve	Elevated	Circular
Y32	Slimy	+ve	Black	Brown	+ve	Elevated	Circular
Y33	Slimy	+ve	White	Black	+ve	Elevated	Circular
Y34	Slimy	+ve	White	Orange	+ve	Elevated	Circular
Y35	Slimy	+ve	Black	Brown	+ve	Elevated	Circular
Y36	Powdery	+ve	White	Orange	+ve	Elevated	Irregular
Y37	Slimy	+ve	White	Orange	+ve	Elevated	Circular
Y38	Slimy	+ve	Black	Black	+ve	Elevated	Circular
Y39	Slimy	+ve	Creamish	Yellow	+ve	Elevated	Circular
Y40	Slimy	+ve	Orange	Orange	+ve	Elevated	Irregular
Y41	Powdery	+ve	Black	Black	+ve	Elevated	Circular
Y42	Powdery	+ve	Orange	Orange	+ve	Elevated	Circular
Y43	Slimy	+ve	Brown	Brown	+ve	Elevated	Circular
Y44	Powdery	+ve	White	Brown	+ve	Elevated	Circular
Y45	Powdery	+ve	Grey	Brown	+ve	Elevated	Irregular
Y46	Powdery	+ve	White	Brown	+ve	Elevated	Circular

The phenotypic characteristics of all the cultures showed different shape, pigmentation, and spore production at maturity. Microscopic study revealed that isolated cultures were Gram positive and different spore structures were analysed by phase contrast microscopy. This study thus results in the exploration of distinct *Actinomyces* isolates from mangrove soil of Mangalore region. These distinct *Actinomyces* isolates were further characterised for various biological activities viz., enzyme screening, and production of biologically active peptides for antimicrobial activity. The *Actinomyces* isolates grown on YEA media were checked for preliminary cytotoxicity activity in induced proliferative yeast cells. The Actinomyces isolates grown on SCNA media when subjected for protein extraction resulted in the identification of bioactive peptides that showed significant antimicrobial activity against test organisms. This study supported the production of biologically active peptides from mangrove soil *Actinomyces* that

could be applicable for treating various ailments and alternate for conventional antibiotics. There are many well-known studies describing the exploration of *Actinomyces* cultures and their secondary metabolites production as source for antibiotic production. A very few reports have been reviewed with regard to protein isolation and their biological activity from *Actinomyces.* The selective growth of *Actinomyces* isolates on YEA media were examined as detailed in Table 3.The isolates were Gram + ve and exhibited spore producing filamentous mycelia. These 46 cultures were further characterized for biological activity viz., antibacterial and cytotoxic activity.

Out of total 87 cultures 46 cultures were from YEA media and 41 cultures are from SCNA media. It is well-known that *Actinomyces* shares the characters of both bacteria and fungi and are referred as 'ray fungi'. Early stage of lifecycle predominantly shows bacterial characteristics and at log growth starts exhibiting fungal filamentous mycelia. This could be a reason or advantage for these microbes to produce a discrete number of bioactive compounds.

From our study, the *Actinomyces* isolates capable of growing selectively on both YEA and SCNA media with morphological appearances grabs our attention by different growth patterns ranging from regular to irregular, filamentous mycelia to slimy, varied spore pattern and coloration were distinguished for culture identification. They were usually grown for 7 days incubation time at 30 ± 2 ^{o}C with sporulation. When the growth of *Actinomyces* isolates in different media viz., KA, IA, YMA, SCNA and YEA media was compared, the SCNA and YEA proved the best media for *Actinomyces* growth.

Table 4.2 Cultural characteristics of mangrove soil *Actinomyces* grown on SCNA media.

***Actinomyces* Isolate**	**Texture**	**Pigmentation**	**aerial mycelia color**	**substratum color**	**Gram staining**	**Height**	**Shape**
S1	Powdery	+ve	White	Brown	+ve	Elevated	Circular
S2	Powdery	+ve	White ash	Brown	+ve	Flat	Pyramidal
S3	Slimy	+ve	White	Brown	+ve	Elevated	Circular
S4	Slimy	+ve	Creamish	Brown	+ve	Elevated	Circular
S5	Powdery	+ve	White ash	Brown	+ve	Elevated	Irregular
S6	Powdery	+ve	White ash	Brown	+ve	Elevated	Circular
S7	Powdery	+ve	White	Creamish	+ve	Elevated	Pyramidal
S8	Powdery	+ve	Ash	Brown	+ve	Elevated	Circular
S9	Slimy	+ve	White	Creamish	+ve	Elevated	Circular
S10	Slimy	+ve	Brown	Black	+ve	Elevated	Circular
S11	Powdery	+ve	White ash	Creamish	+ve	Elevated	Irregular
S12	Slimy	+ve	Yellow	Creamish	+ve	Elevated	Irregular
S13	Powdery	+ve	Ash	Black	+ve	Elevated	Circular
S14	Powdery	+ve	Ash	Black	+ve	Elevated	Circular
S15	Slimy	+ve	Grey ash	Black	+ve	Elevated	Circular
S16	Punctiform	+ve	White	Black	+ve	Elevated	Circular
S17	Powdery	+ve	White	Brown	+ve	Elevated	Irregular
S18	Slimy	+ve	Yellow	Creamish	+ve	Elevated	Circular
S19	Slimy	+ve	Yellow	Brown	+ve	Elevated	Circular
S20	Powdery	+ve	White ash	Creamish	+ve	Elevated	Circular
S21	Powdery	+ve	White ash	Black	+ve	Elevated	Circular
S22	Powdery	+ve	Yellow	Creamish	+ve	Flat	Irregular
S23	Powdery	+ve	Creamish	Brown	+ve	Elevated	Circular
S24	Slimy	+ve	Brown	Brown	+ve	Elevated	T Shaped /Irregular
S25	Slimy	+ve	Yellow	Creamish	+ve	Elevated	Circular
S26	Slimy	+ve	Creamish	Creamish	+ve	Elevated	Irregular
S27	Powdery	+ve	White ash	Brown	+ve	Elevated	Circular
S28	Powdery	+ve	White	Black	+ve	Elevated	Circular
S29	Slimy	+ve	Pale Yellow	Creamish	+ve	Elevated	Irregular
S30	Slimy	+ve	White ash	Colorless	+ve	Elevated	Irregular

S31	Slimy	+ve	Creamish	Colorless	+ve	Elevated	Circular
S32	Slimy	+ve	Yellow	Creamish	+ve	Elevated	Circular
S33	Powdery	+ve	Creamish	White	+ve	Elevated	Circular
S34	Slimy	+ve	Black ash	Black	+ve	Elevated	Circular
S35	Powdery	+ve	Brown	Brown	+ve	Elevated	Circular
S36	Powdery	+ve	Creamish	Creamish	+ve	Elevated	Circular
S37	Slimy	+ve	Creamish	Creamish	+ve	Elevated	Irregular
S38	Powdery	+ve	Light Yellow	Creamish	+ve	Elevated	Circular
S39	Powdery	+ve	White	Brown	+ve	Elevated	Circular
S40	Powdery	+ve	Black	Black	+ve	Elevated	Circular
S41	Slimy	+ve	Grey	Brown	+ve	Elevated	Circular

The SCNA cultures also showed varied growth patterns and same parameters were used for both SCNA and YEA media cultures. The *Actinomyces* isolates grown on SCNA media were characterized for colony morphology and staining as detailed in **Table 4.2.**

The previous study described successful isolation of *Actinomyces* isolates using SCNA media. SCNA media was efficient to isolate 100 isolates from great Indian Thar desert. The cultured isolates were potential producers of antibiotics (Tiwari et al., 2015; Tiwari and Gupta, 2012). Another study from Western Ghats of Tamilnadu successfully isolated 106 distinct *Actinomyces* isolates on SCNA media and showed antimicrobial activity against wide spectrum of pathogens (Ganesan et al., 2017). The isolation of novel *Actinomyces* isolates were reported from Japan using yeast extract media and characterized as *Actinomadura*, *Streptosporangium* and *Microbispora* species (Hamaki et al., 2005). Another study states that use of yeast extract media successfully explores *Actinomyces* isolates from beehives of Thailand resulted *Nocardiopsis*, *Actinomadura* and *Nonomurae sp* (Promnuan et al., 2009).

The isolation of thermophilic and mesophilic *Actinomyces* isolates from Mouldy hay resulted *Micromonospora vulgaris, Thermopolyspora polyspora, T. glauca, Streptomyces thermoviolaceus, S. fradiae, S. griseoflavus, S. olivaceus*and *S. griseus* (Corbaz et al., 1963).

4.2 Extraction of mangrove soil *Actinomyces* protein

The protein extraction was initiated after mass culturing of respective *Actinomyces* strains along with a supplementation of 1 % soya peptone. The cultured biomass yielded significant amount of intracellular protein extract. This protein extraction was followed according to Bhaduri1983 that explained simple and rapid method for disruption of bacteria for protein studies could able to yield significant amount of protein compared to conventional homogenization methods. The obtained protein was estimated using Biuret's method and yielded an average of 14-16 mg of intracellular protein from 1.0 g of *Actinomyces* culture biomass. The obtained crude protein was subjected for preliminary antimicrobial activity.

The extracted protein were subjected for antimicrobial activity, about 50 μg of crude protein was used for antibacterial activity studies against common test organisms like *Escherichia coli* (ATCC8739), *Klebsiella pneumoniae* (ATCC9621), *Pseudomonas aeruginosa* (ATCC9027) and *Staphylococcus aureus* (ATCC6538P).Potential *Actinomyces* protein were further analysed by using SDS-PAGE electrophoresis after silver nitrate staining. Further, differential protein purification method by chromatography techniques for purification and identification of bioactive peptides will be discussed in the subsequent chapter.

Figure 4.8 SDS-PAGE electropherogram for *Actinomyces* isolates protein.

Actinomyces intracellular protein of 20 µg from *Actinomyces* isolates S6, S17, S23 and S24that showed effective antimicrobial activity was analysed by SDS-PAGE electropherogram. After electrophoretic run the SDS-PAGE gel was stained with 2.0 % silver nitrate according Blum et al., 1987.In brief, Blum's silver staining allowed for 30 minute detection of proteins in Polyacrylamide gels. This SDS-PAGE analysis gave a key indication for isolation of protein from *Actinomyces* isolates. The previous study of Nikolaitchouk et al., 2000 successfully elucidated the species characterization using by PAGE method (Nikolaitchouk et al., 2000). Whereas 2-DE was first attempted to analyze the proteome profile of *Streptomyces avermitilis* during batch fermentations in complex medium(Yin et al., 2008).

The antimicrobial activity of *Actinomyces* isolates grown on YEA media were checked against *E.coli, Klebsiella pneumoniae Pseudomonas aeruginosa* and *Staphylococcus aureus* as detailed in **Table 4.3.**The YEA cultures Y9 and Y12 isolates showed significant activity against *Staphylococcus aureus. Staphylococcus aureus*is a causative organism for endocarditis, metastatic infection, and sepsis syndrome(Lowy, 1998). It contributes to the activation of endovascular disease development. The Y36, Y37, Y 39, Y40, Y41, Y42 and Y45 showed potential antibacterial activity against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is causative microbe for chronic obstructive pulmonary disease (COPD) and cystic fibrosis(Kotiranta et al., 2000). Culture Y36, Y45 and Y46 showed efficient activity against *Klebsiella pneumoniae. Klebsiella pneumoniae* is a causative organism for urinary tract infection (UTI), pneumonia, intra-abdominal infection, bloodstream infection (BSI), meningitis and pyogenic liver abscess (PLA)(Lederman and Crum, 2005). Isolates Y17, Y18, Y19 and Y20 were active against *Escherichia coli. Escherichia coli* is a causes diarrhea, urinary tract

infections, respiratory illness and pneumonia, and other illnesses (Auer et al., 2009; Goldwater and Bettelheim, 2012; Gould, 2010).

Table 4.3Antimicrobial activity of *Actinomyces* protein extract from Y1 to Y46.

***Actinomyces* isolate protein**	**Zone of Inhibition (mm)**			
	Escherichia coli	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
Y1	-	7 ± 0.3	-	-
Y2	6 ± 0.3	6 ± 0.3	-	-
Y3	8 ± 0.3	-	6 ± 0.3	6 ± 0.3
Y4	6 ± 0.3	6 ± 0.3	-	-
Y5	-	7 ± 0.6	6 ± 0.3	7 ± 0.3
Y6	-	6 ± 0.3	6 ± 0.3	6 ± 0.3
Y7	7 ± 0.3	-	8 ± 0.6	-
Y8	8 ± 0.6	6 ± 0.3	-	6 ± 0.3
Y9	6 ± 0.3	6 ± 0.6	7 ± 0.6	**11± 0.6**
Y10	8 ± 0.3	6 ± 0.3	6 ± 0.3	7 ± 0.3
Y11	6 ± 0.3	6 ± 0.3	-	7 ± 0.6
Y12	-	-	-	**13± 0.6**
Y13	-	-	-	-
Y14	-	8.0	-	-
Y15	7 ± 0.6	8 ± 0.3	-	6 ± 0.3
Y16	6 ± 0.3	8 ± 0.6	-	-
Y17	**12 ± 0.3**	9 ± 0.6	6 ± 0.3	-
Y18	**16 ± 0.3**	7 ± 0.3	-	-
Y19	**12± 0.6**	7 ± 0.3	7 ± 0.3	-
Y20	**10± 0.6**	6 ± 0.3	-	8 ± 0.6
Y21	6 ± 0.3	6 ± 0.6	8 ± 0.6	-
Y22	7 ± 0.6	6 ± 0.3	-	-
Y23	-	8 ± 0.3	-	6 ± 0.6
Y24	-	6 ± 0.6	-	-
Y25	6 ± 0.6	6 ± 0.6	-	6 ± 0.6
Y26	8 ± 0.6	-	6 ± 0.3	6 ± 0.3
Y27	6 ± 0.3	6 ± 0.6	-	-
Y28	6 ± 0.3	6 ± 0.6	-	6 ± 0.3
Y29	7 ± 0.3	-	-	-
Y30	7 ± 0.3	6 ± 0.6	-	-
Y31	8 ± 0.3	-	-	7 ± 0.3
Y32	6 ± 0.3	8 ± 0.6	-	7 ± 0.6
Y33	-	6 ± 0.3	-	7 ± 0.3

Y34	-	6 ± 0.3	-	6 ± 0.6
Y35	-	7 ± 0.3	-	-
Y36	-	**10± 0.6**	**14.0**	7 ± 0.3
Y37	6 ± 0.3	6 ± 0.3	**15.0**	-
Y38	-	7 ± 0.3	6 ± 0.3	-
Y39	-	7 ± 0.3	**17± 0.3**	-
Y40	6 ± 0.3	8 ± 0.3	**15± 0.3**	-
Y41	6 ± 0.3	6 ± 0.3	**14± 0.6**	6 ± 0.3
Y42	-	-	**13± 0.6**	8 ± 0.3
Y43	-	6 ± 0.3	-	6 ± 0.6
Y44	6 ± 0.3	-	-	6 ± 0.3
Y45	7 ± 0.6	**15 ± 0.3**	**16± 0.6**	7 ± 0.3
Y46	6 ± 0.3	**13 ± 0.6**	8 ± 0.3	8 ± 0.3
Streptomycin	10 ± 0.3	9 ± 0.3	9 ± 0.3	10 ± 0.3

4.3 Cytotoxic Activity

The cytotoxicity activity was performed using UV radiation as physical mutagen and 5 % H_2O_2 as chemical mutagens that were induced into yeast cells transforming them into highly proliferative cells. The protein from each of the isolates when assayed for cytotoxicity against normal yeast cellular proliferation and abnormal (Chemical & Physical) mutagen treated yeast cells showed significant cytotoxicity against highly proliferative yeast cells detailed in **Table 4.4.**The results of cytotoxicity assay in yeast cells, upon treatment with protein extracts the isolates Y3, Y6, Y14, Y15, Y18, Y33, Y37, Y38, Y39, Y41, Y45, Y46 showed significant cytotoxicity against highly proliferative yeast cells. The *Actinomyces* protein cytotoxic activity against UV induced yeast proliferative cells.

This result thus attributes the microbial protein having antimicrobial activity also exerts antiproliferative activity and thus can be implicated towards the identification of anticancer peptides (ACP's). Among 46 YEA Actinomyces isolates, 17 isolates showed efficient antimicrobial and cytotoxic activity as detailed in **Table 4.4.**This result gives insight

understanding about the protein activities that are antimicrobial will also act as anticancer peptides.

The bioactive peptides in the present work considered as antimicrobial peptides were checked for cytotoxic activity against *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* serves as a eukaryotic cell model that shares genetic homology with human genetic system (Bjornsti et al., 1994). Here we initiated the extracted antimicrobial peptides from *Actinomyces* for yeast cell cytotoxicity testing after yeast cell transformation with treatment using physical and chemical mutagens. This work substantiated to determine antimicrobial peptide also serving as anticancer peptides. Therefore, around seventeen *Actinomyces* isolate peptides in our study have shown antimicrobial and cytotoxic activity.

Table 4.4Comparison of percentage cytotoxicity and antimicrobial activity of protein extracts of isolates.

Actinomycetes Isolates	Percentage Cytotoxicity		Antimicrobial Activity
	UV induced Proliferation	5%H_2O_2 induced Proliferation	
Y3	40.77	36.33	+++
Y6	56	39.88	+++
Y7	23.7	8.93	++
Y11	27	39.28	+++
Y14	53.3	39.88	+
Y15	40.77	38.21	+++
Y18	38.84	43	++
Y19	28.9	25.71	+++
Y25	14.9	16.37	+++
Y32	37.86	29.58	+++
Y37	58.3	-	+++
Y38	38.8	-	++
Y39	50.79	18.45	++
Y41	50.79	36.73	++++
Y42	28.59	40.48	++
Y45	56.6	53.57	++++
Y46	62.53	43.43	++++

++++ (shown activity against four test organisms)
+++ (shown activity against three test organisms)
++ (shown activity against two test organisms)

The protein compound from *Actinomyces* isolates Y3, Y6, Y11, Y15, Y19have shown significant antimicrobial effect against three test organisms and also more than 25 % of cytotoxic activity. However, Actinomycete isolate Y41, Y45, Y46, have shown significant antimicrobial effect against four test organisms and with more than 50 % cytotoxic activity. Therefore, these three actinomycete isolates are thus beneficial for exploring novel peptides with target specific activity.

4.4 Antimicrobial activity for SCNA cultures

The 41 *Actinomyces* isolates sub-cultured from SCNA media were grown on starch casein nitrate broth (SCN). Incorporating 1 % soya peptone could able to produce more isolate biomass thus speculating in the subsequent production of intracellular protein. The protein extraction was followed according to Bhaduri as discussed above. The protein extract was estimated by Biuret's method and about 14-16 mg of protein yield was obtained from 1 gm of *Actinomyces* isolate biomass. An about 50 µg of extracted protein was pipetted to nutrient agar (NA) plates into labeled wells to analyse antibacterial activity against eight inoculated test cultures such as *Escherichia coli* (ATCC8739), *Pseudomonas aeruginosa* (ATCC9027), *Klebsiella pneumoniae* (ATCC9621), *Staphylococcus aureus* (ATCC6538P), *Bacillus cereus* (ATCC10876), *Proteus vulgaris* (ATCC13315), *Salmonella typhimurium* (ATCC23564), and *Enterobacter aerogenes* (ATCC13048) procured from NCIM (NCL, Pune). After 24 hr of incubation period, zone of inhibition was measured and detailed in **Table 4.5.**

The results proved that SCNA *Actinomyces* isolates can able to produce peptides having antimicrobial activity that can be referred further as bioactive antimicrobial peptides (AMP's). Peptides which are effective to retard the growth of other microbes are called as antimicrobial peptides. These peptides produced by SCNA *Actinomyces* isolates showed effective inhibition

against *B. cereus P. vulgaris S. aureus and P. aeruginosa*. The protein extracts from *Actinomyces* isolates S4, S12, S14, S18 and S30 showed no activity against eight test pathogens. The protein extracts from *Actinomyces* isolates S15, S16, S26, S27, S28, and S37 showed effectiveness against *Salmonella typhimurium*. The protein extracts from *Actinomyces* isolates S2, S9, S11, S20, S22, S34 and S38 showed effective activity against *Proteus vulgaris and Salmonella typhimurium*. The protein extracts from *Actinomyces* isolates S3, S7, S8, S10, S19, S21, S25, S31, S32, S35, S39, S40 and S41 were active against *Bacillus cereus, Proteus vulgaris* and *Salmonella typhimurium*.

Table 4.5 Antibacterial activity of protein extracted from SCNA *Actinomyces* isolates.

Isolate	***B. cereus***	***K pneumoniae***	***E aerogenes***	***P vulgaris***	***E coli***	***S typhimurium***	***S aureus***	***P aeruginosa***
S1	14±0.6	--	--	14±0.6	--	--	17±0.6	14±0.6
S2	--	--	--	13±0.6	--	--	13±0.6	--
S3	10±0.6	--	--	--	--	--	10±0.6	11±0.6
S4	--	--	--	--	--	--	--	--
S5	12±0.6	--	--	14±0.6	--	--	17±0.6	10±0.2
S6	**15±1**	--	--	**18±0.6**	--	**12±0.6**	**18±0.6**	**14±0.6**
S7	10±0.6	--	--	--	--	--	14±0.1	11±1
S8	13±0.6	--	--	--	--	8±0.6	19±0.6	--
S9	14±0.6	--	--	--	--	--	16±0.6	--
S10	12±0.6	--	--	--	--	--	15±0.3	11±0.6
S11	--	9 ±0.6	--	--	--	--	--	8±0.6
S12	--	--	--	--	--	--	--	--
S14	--	--	--	--	--	--	--	--

S15	--	8±0.6	--	--	--	--	--	--
S16	--	8±0.6	--	--	--	--	--	--
S17	**19±0.6**	--	--	**22±0.6**	--	**19±0.6**	**20±0.6**	**16±0.6**
S18	--	--	--	--	--	--	--	--
S19	--	--	--	14±0.6	13±0.6	--	10±0.6	--
S20	--	--	--	12±0.6	14±0.1	--	--	--
S21	10±0.6	--	--	16±1.0	--	--	10±0.6	--
S22	--	--	--	8 ±0.3	13±0.3	--	--	--
S23	**10±0.6**	**--**	**--**	**22±1.0**	**--**	**14±0.6**	**15±0.6**	**13±0.6**
S24	**19±0.6**	**--**	**--**	**24±1.0**	**--**	**19±0.6**	**19±0.6**	**14±0.6**
S25	--	--	--	11±0.6	--	8±1.0	10±1.0	--
S26	--	--	--	--	--	8±0.6	--	--
S27	--	--	--	--	--	8±0.6	--	--
S28	--	--	--	--	--	8±0.6	--	--
S29	15±1.0	--	--	16±0.6	--	--	18±1.0	12±0.6
S30	--	--	--	--	--	--	--	--
S31	11±0.6	--	--	--	--	--	12±0.6	8±0.6
S32	10±0.6	--	--	--	--	12±0.6	14±0.6	--
S33	--	--	--	--	--	--	--	--
S34	11±0.6	--	--	--	--	--	15±0.6	--
S35	--	--	--	8±0.6	--	10±0.3	--	10±0.3
S36	10±0.3	--	--	11±0.6	--	10±0.3	--	11±0.6
S37	--	--	--	--	--	8±0.3	--	--

S38	--	--	--	11±0.3	--	8±0.3	--	--
S39	8±0.6	--	--	14±0.3	--	12±0.6	--	--
S40	10±0.6	--	--	12±0.6	--	--	13±0.6	--
S41	11±0.3	--	--	15±0.3	--	10±0.6	--	--
Streptomycin	6 ± 0.6	7 ± 0.6	6 ± 0.6	5 ± 0.6	5 ± 0.6	6 ± 0.6	7 ± 0.6	5 ± 0.6

The protein extracts from *Actinomyces* isolates S1, S4, S29 and S36 showed inhibition against *Bacillus cereus, Proteus vulgaris, Salmonella typhimurium* and *Pseudomonas aeruginosa*. The protein extracts from *Actinomyces* isolates **S6, S17, S23** and **S24**were effective against five test cultures viz., *Bacillus cereus, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus* and *Pseudomonas aerogenes*. Therefore, the *Actinomyces* isolates **S6, S17, S23** and **S24** were the prominent *Actinomyces* cultures and their intracellular protein extract showed effective inhibition against five test pathogens. Bioactive peptide obtained from four *Actinomyces* isolates showed significant activity against Gram positive bacteria viz., *S. aureus* and *B. cereus.* Clinically *S. aureus*is a causative organism for endocarditis, metastatic infection, and sepsis syndrome. Itcontributes to the activation of endovascular disease development (Lowy, 1998). *B. cereus* causes diarrhea, gastrointestinal pain, and emetic syndrome by secretion of enterotoxins (Kotiranta et al., 2000).

These four *Actinomyces* protein was effective against Gram negative bacteria *P. vulgaris, S. typhimurium,* and *P. aeruginosa. P. vulgaris* is a human opportunistic pathogen, it has been linked to infantile enteritis and colitis, as well as a rise in mortality rates (Neter and Farrar, 1943). *S. typhimurium* is primarily responsible for causing typhoid fever and salmonellosis in humans (Gilman and Hornick, 1976; Moffatt et al., 2016). *P. aeruginosa* is involved in chronic

obstructive pulmonary disease (COPD) and cystic fibrosis (Murphy et al., 2008).These four SCNA *Actinomyces* isolates were highly efficient compared to YEA isolates. YEA isolates showed moderate effectiveness against four test pathogens. Hence, these four mangrove soil *Actinomyces* isolates **S6, S17**, **S23** and **S24**wereconsidered to be prominent cultures and therefore selected for further studies on identification and characterization of their bioactive peptides. The antimicrobial activity of the bioactive peptides from selected four *Actinomyces* protein extract as performed by well diffusion method is shown in **Figure 4.9-4.12.** The antimicrobial efficiency of the bioactive protein extracts were compared with standard antibiotics streptomycin 10 µgas depicted in **Graphs 4.3-4.6**.

A vast literatures available for efficiency of secondary metabolites and production of antibiotics from *Actinomyces* (Tiwari and Gupta, 2012). The primary metabolites are the important biomolecules having many biological activities. The proteins are biomolecules and considered as building blocks of life with distinguished biological functions. The pathways of any biological functions are regulated by an array of protein molecules. A wide literature studies have reported on microbial protein/peptides with various biological activities from bacteria and fungi (Collin and Maxwell, 2019; Elayaraja et al., 2014). Few studies are available on protein extracted from *Actinomyces* group (Alapati and Muvva, 2013; Yin et al., 2008).The *Actinomyces* shares characters of both bacteria and fungi as a result these *Actinomyces* can act as a host for synthesis of large number of proteins from *Streptomyces* group.

The *Actinomyces* isolate S6 on SCNA media exhibited white ash colored filamentous mycelia producing grey color spores at maturity, positive pigmentation and Gram +ve as detailed in **Table 4.6**. The isolate was subjected to morphological studies by aid of inverted phase contrast microscope and ZEISS FESEM microscopy. The culture showed filamentous mycelia possessing

spirally coiled spore chain. Each spore visualized as elongated cylindrical hairy appearance with curved edges as shown in **Figure 4.13.**

The *Actinomyces* isolate S17was observed as white colored filamentous mycelia producing ash colored spores at maturity, positive pigmentation on SCNA media with Gram staining +ve. The microscopic characteristics were observed for filamentous mycelia possessing straight spore chain. Spores visualized as elongated rod shaped smooth surfaced spore with curved edges as shown in **Figure 4.14.**

Actinomyces isolate S23 showed creamish white colored filamentous mycelia producing grey colored spores at maturity, positive pigmentation on SCNA media with Gram staining +ve and microscopically characterized as filamentous mycelia possessing spirally coiled spore chain bearing cylindrical hairy spore with curved edges as shown in **Figure 4.15.**

The *Actinomyces* strain S24 showed creamish brown colored filamentous mycelia producing grey colored pointed spores at maturity, positive pigmentation on SCNA media with Gram +ve**.** This culture showed distinct micro morphology compared to other isolates. FESEM revealed filamentous long slender mycelia bearing bunch of spores as sporangia. Each spore were observed as spherical spiny ball like appearance as shown in **Figure 4.16.**

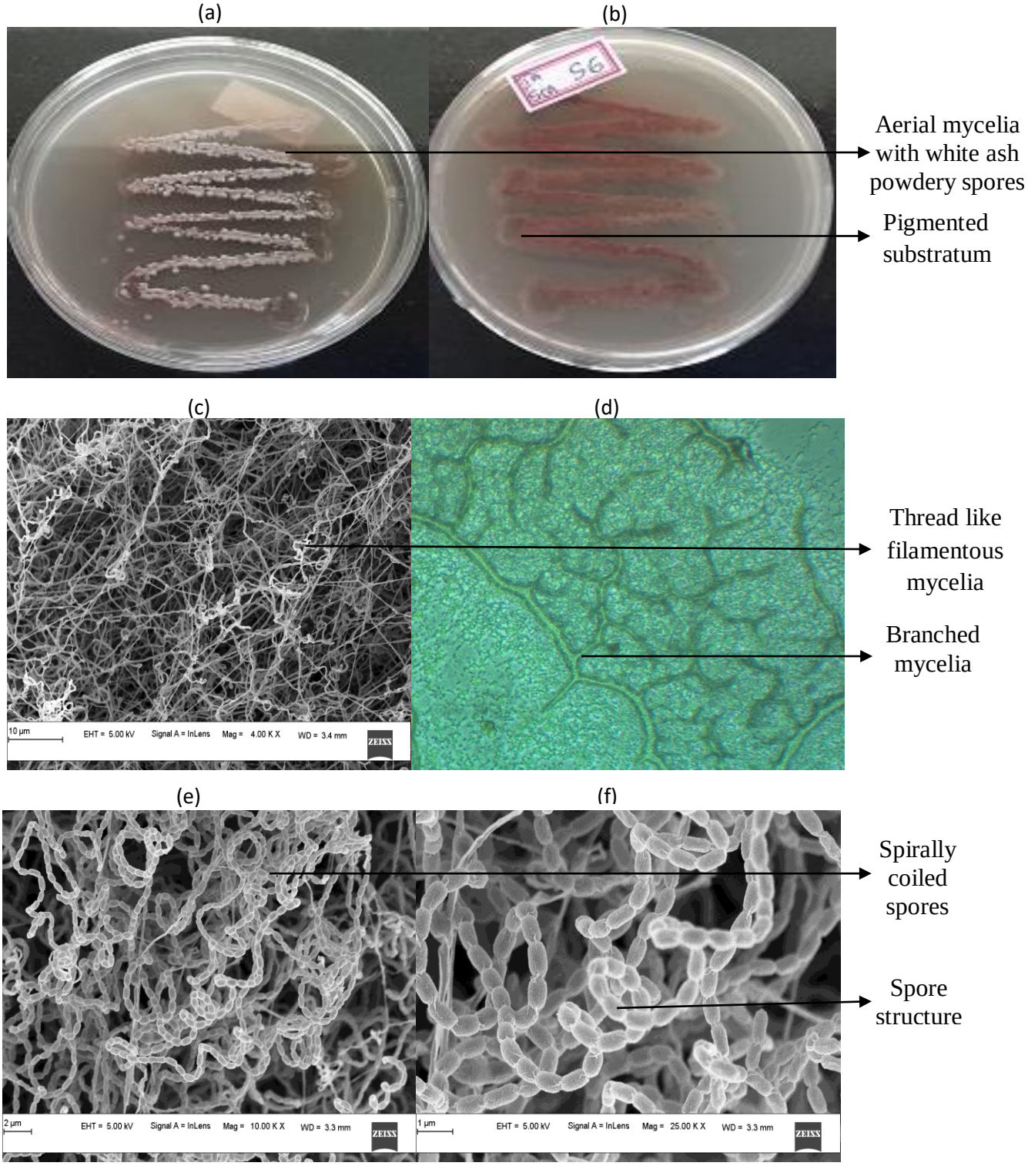

Figure4.13 Cultural characteristics of *Actinomyces* isolate S6. (a) Front view of isolate (b) Rear view of isolate (c) Mycelia observations under FESEM (d) Phase contrast microscopic analysis (e) Mycelia along with spore analysis under FESEM (f) Spore structure analysis using FESEM.

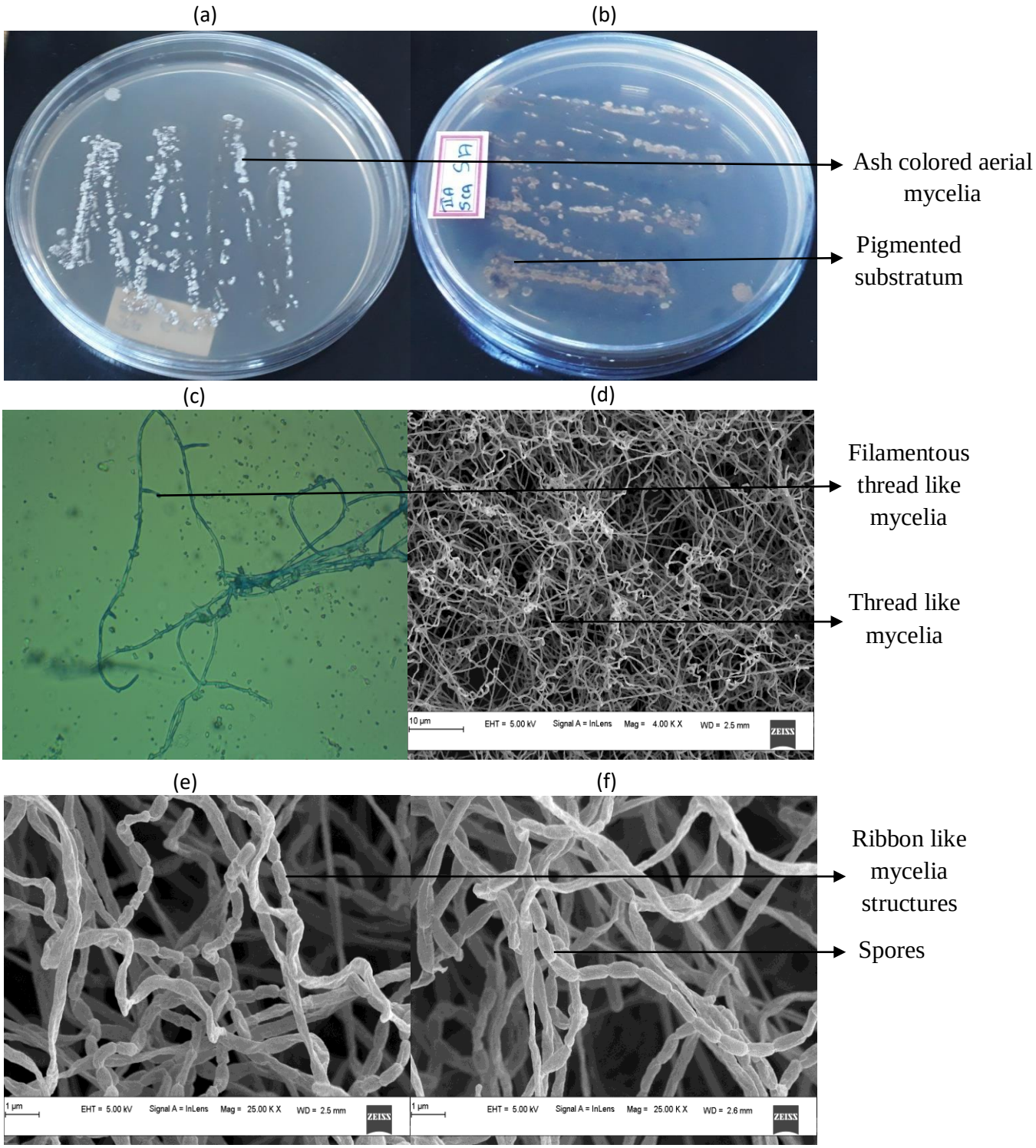
(a)
(b)
Ash colored aerial mycelia
Pigmented substratum
(c)
(d)
Filamentous thread like mycelia
Thread like mycelia
10 µm EHT = 5.00 kV Signal A = InLens Mag = 4.00 K X WD = 2.5 mm ZEISS
(e)
(f)
Ribbon like mycelia structures
Spores
1 µm EHT = 5.00 kV Signal A = InLens Mag = 25.00 K X WD = 2.5 mm ZEISS
1 µm EHT = 5.00 kV Signal A = InLens Mag = 25.00 K X WD = 2.6 mm ZEISS

Figure 4.14 Cultural characteristics of *Actinomyces* isolate S17. (a) Front view of isolate (b) Rear view of isolate (c) Mycelia observations under FESEM (d) Phase contrast microscopic analysis (e) Mycelia along with spore analysis under FESEM (f) Spore structure analysis using FESEM.

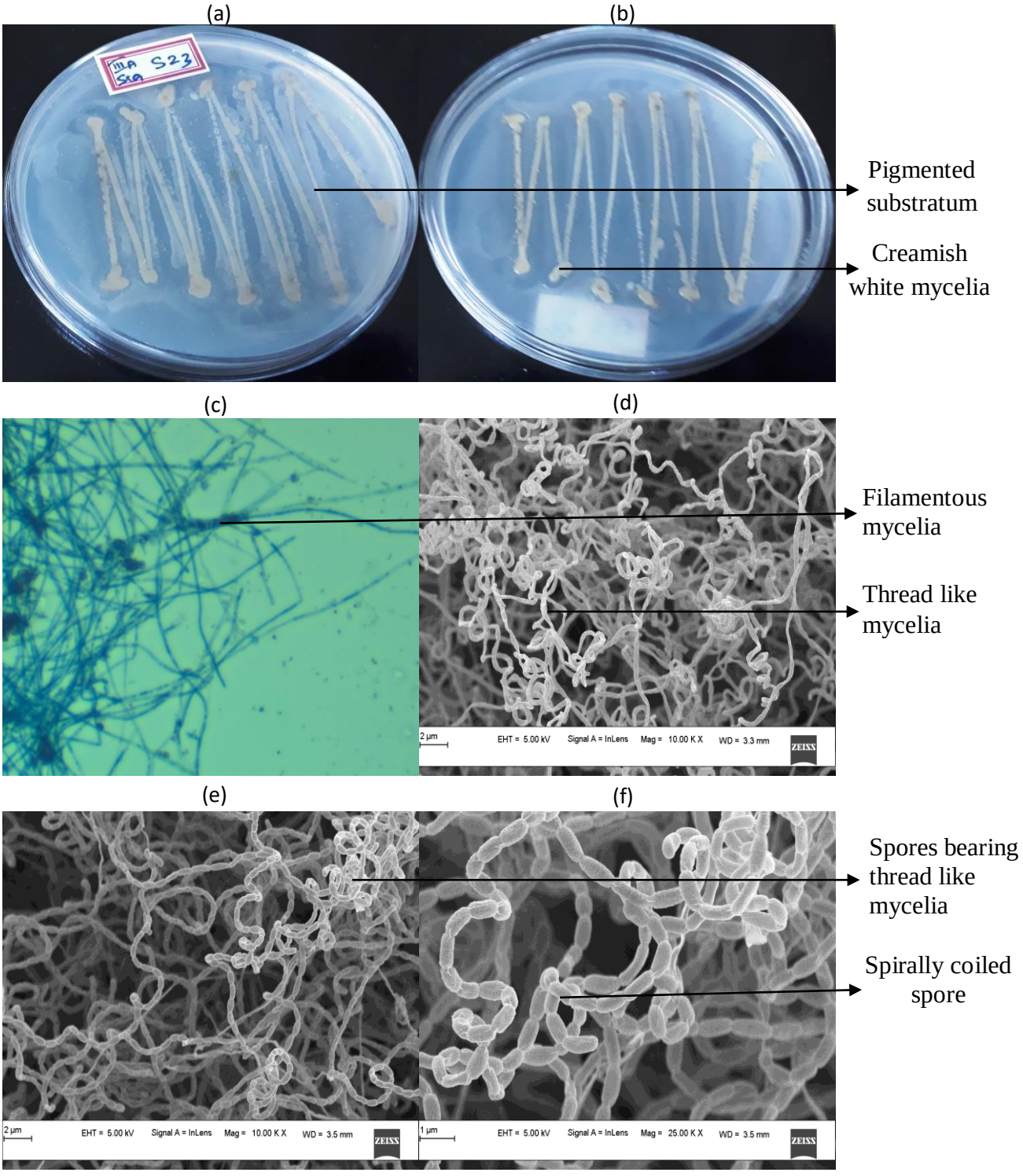

Figure 4.15Cultural characteristics of *Actinomyces* isolate S23. (a) Rear view of isolate (b) Front view of isolate (c) Phase contrast microscopic analysis (d) Mycelia observations under FESEM (e) Mycelia along with spore analysis under FESEM (f) Spore structure analysis using FESEM.

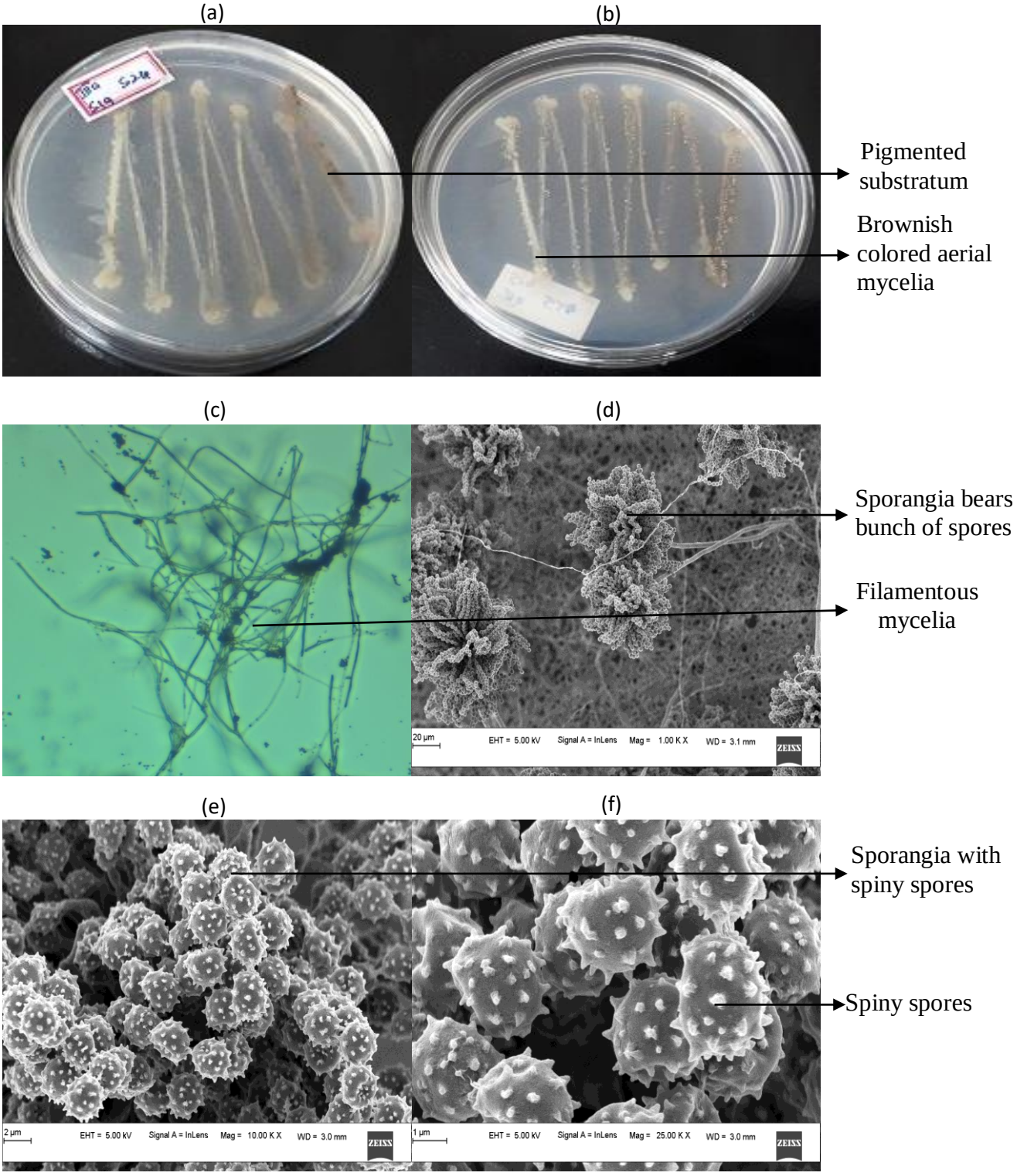

Figure 4.16Cultural characteristics of *Actinomyces* isolate S23. (a) Rear view of isolate (b) Front view of isolate (c) Phase contrast microscopic analysis (d) Mycelia observations under FESEM (e) Mycelia along with spore analysis under FESEM (f) Spore structure analysis using FESEM.

Table4.6 Phenotypic characteristics of *Actinomyces* isolates on different media.

Isolate	Media	Growth	Front view	Rear view	Pigment	Spores
S6	Sucrose peptone agar	Moderate	Cream	Creamish white	-	No
S17		Good	Creamish	Creamish	-	White
S23		No	-	-	-	No
S24		Good	Cream	Creamish	-	White powdery
S6	Glucose luecine agar	Good	Cream	White	-	Black
S17		No	-	-	-	No
S23		No			-	
S24		Good	Creamish	Creamish	-	Black
S6	Nutrient agar	Good	Creamish	Creamish	-	-
S17		Good	Cream	Cream	-	-
S23		No	-	-	-	-
S24		Good	Cream	Cream	-	Grey Powdery
S6	Malt extract agar	Moderate	Creamish white	Creamish white	-	No
S17		Moderate	Cream	Cream	-	No
S23		No	-	-	-	No
S24		Good	Creamish	Creamish	-	Grey powdery
S6	Yeast extract agar	Good	Cream	Cream	-	White
S17		Good	Cream	Cream	-	No
S23		Good	Cream	Creamish white	-	No
S24		Good	Cream	Cream	-	White powdery
S6	**Starch casein nitrate agar**	**Excellent**	**White ash**	**Brown**	**+**	**Grey**
S17		**Excellent**	**White**	**Brown**	**+**	**Ash**
S23		**Excellent**	**Brown**	**Black**	**+**	**Grey**
S24		**Excellent**	**Creamish**	**Brown**	**+**	**Grey**

4.5 Molecular sequencing of *Actinomyces* isolates

The bioactive peptide producing Actinomyces isolate S6, S17, S23, and S24 were selected for molecular identification. DNA was isolated from each *Actinomyces* isolates as performed using CTAB. The purity of DNA was checked and quantified after the isolated DNA was subjected to purification according to Himedia DNA purification kit. The DNA was further subjected to PCR amplification. The primer designing was done using primer designing tool of NCBI databases and the forward primer27F 5'- AGAGTTTGATCCTGGCTCAG-3' reverse primer 1492R 5'-GGTTACCTTGTTACGACTT-3' were selected for *Actinomyces* identification. Amplified DNA detected on Agarose gel electrophores is showed in **Figure 4.17**was subjected for 16s rRNA

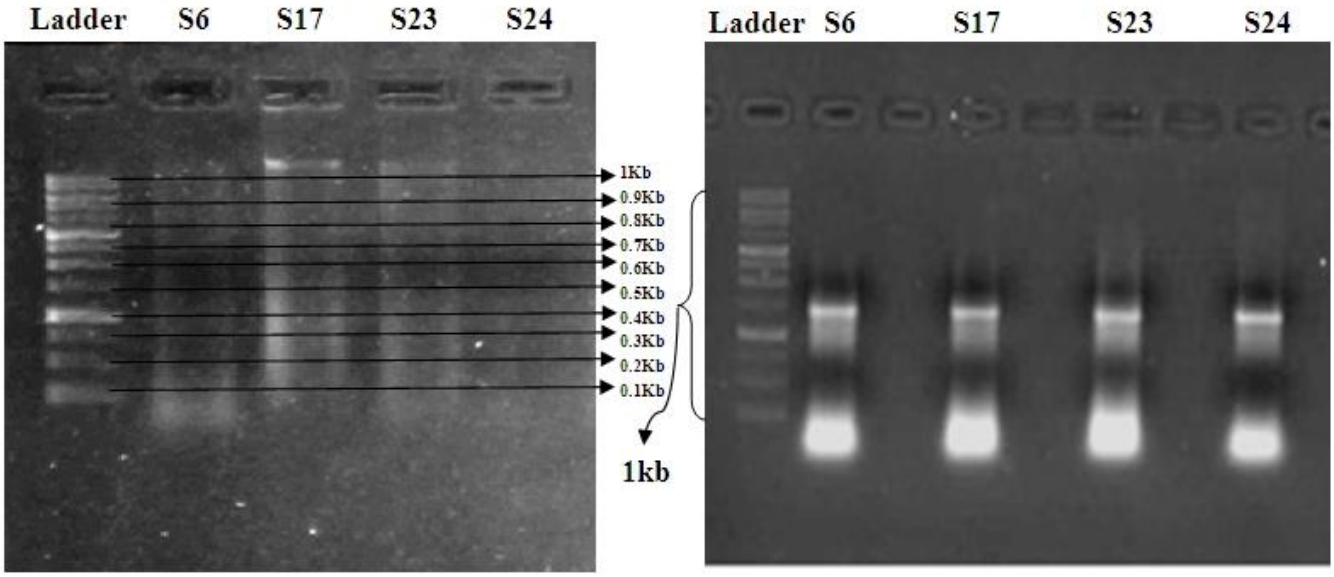

Figure4.17(a)Agarose gel electrophoresis for isolated DNA. (b) PCR amplified DNA from *Actinomyces* isolates.

gene sequencing. The DNA amplicons was subjected for 16s rRNA gene sequencing, the sequences were analysed using BioEdit application that picks a quality sequence and resulted sequence was used for nucleotide BLAST at NCBI server. The four *Actinomyces* isolates were identified that showed maximum resemblances to the genus of *Streptomyces, Actinomyces* and *Arthrobacter*. These sequences were deposited to GenBank nucleotide sequence collection

databases provided with an accession numbers for each *Actinomyces* isolate. The phylogenetic tree constructed using MEGA X aligned sequence with aid of muscle program along with neighbor joining method with bootstrap values of 1000 replicons. The S6 *Actinomyces* isolate was identified as *Glutamicibacter mysorens* with GenBank accession no. MW647910.1and phylogenetic tree constructed as shown **Figure 4.18**withmaximum likelihood species listed in **Table 4.7.**

>S6
TTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGT
GACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTGGAGGCAGCAGTGGGGA
ATATTGCACAATGGGCGGAAGCCTGATGCAGCGACCCGCGTGAGGGATGACGGCCT
TCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAA
GAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTT
ATCCGGATTTATTGGGCGTAAATCGTAGGCGGTTTGTCGCGTCTGCCGTGAAAGTCC
GACTCAACCTCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAGGGGAGAC
TGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCG
AAGGCAGGTCTCTGGGCATTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAAC
AGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGGA
CATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCGCCTGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATG
CGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTCATGTGCCAGACCGCT
CCAGAGATGGGGTTTCCCTTCGGGGCTGGTTCACAGGTGGTGCATGGTTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCCATGT
TGCCAGCACGTAGTGGTGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCTATGTCTTGGGCTTCACGCATGCTACAAT
GGCCGGCAATGGGTTGCGATACTGTGAGGTGGAGCTAATCCCTAAAAGCCGGTCTC
AGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCA
GCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCA
CGAGTTGGTAACA

Hit (Genus): ***Arthrobacter,*** Scientific name: ***Glutamicibacter mysorens*** **JX184904.1**

Query coverage: **100%,** E value: 0.0

Percentage identity: **98.19**

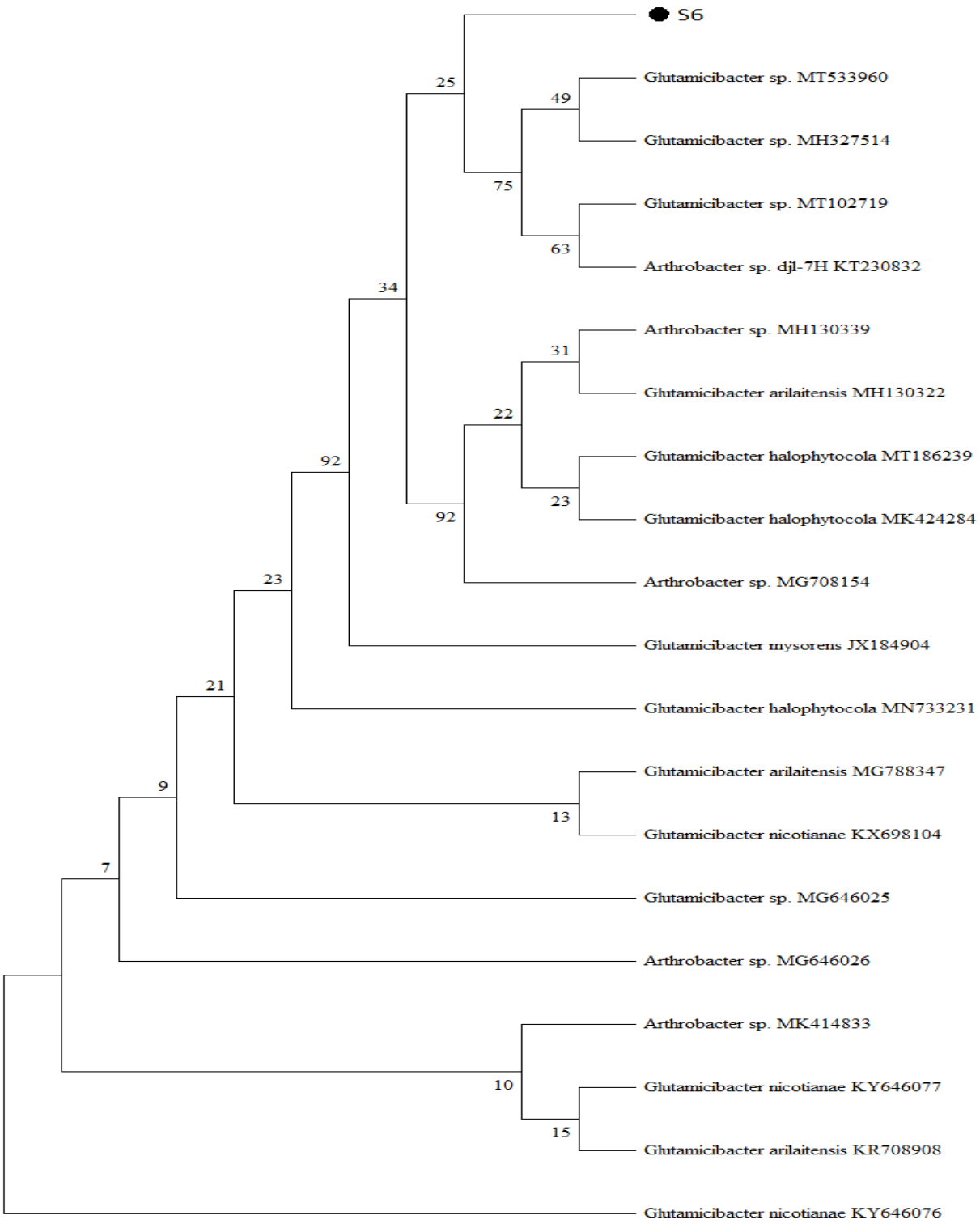

Figure4.18 Phylogenetic tree of*GlutamicibactermysorensYKIKM.MU* (S6) using Neighbor-Joining method.

Table 4.7 Maximum relatedness species to *Glutamicibacter mysorens* YKIKM.MU

Description	Scientific Name	Query Cover	E value	Per. Ident	Acc. Len	Accession
Arthrobacter mysorens strain 1	*Glutamicibacter mysorens*	100%	0.0	98.19	1201	JX184904.1
Glutamicibacter sp. strain S_C_o-B10	*Glutamicibacter sp.*	100%	0.0	98.02	1348	MT533960.1
Glutamicibacter mishrai strain S5-52	*Glutamicibacter mishrai*	100%	0.0	98.02	3570747	CP032549.1
Arthrobacter sp. strain EB53	*Arthrobacter sp.*	100%	0.0	98.02	1389	MH130339.1
Glutamicibacter arilaitensis strain EB36	*Glutamicibacter arilaitensis*	100%	0.0	98.02	1382	MH130322.1
Glutamicibacter halophytocola strain ST67	*Glutamicibacter halophytocola*	100%	0.0	98.02	1524	MT186239.1
Glutamicibacter sp. strain Agri-11	*Glutamicibacter sp.*	100%	0.0	98.02	1394	MT102719.1
Glutamicibacter halophytocola strain WT14	*Glutamicibacter halophytocola*	100%	0.0	98.02	1394	MN733231.1
Glutamicibacter halophytocola strain DR408 chromosome,	*Glutamicibacter halophytocola*	100%	0.0	98.02	3770186	CP042260.1
Glutamicibacter sp. strain SMB32	*Glutamicibacter sp.*	100%	0.0	98.02	1417	MH327514.1
Arthrobacter sp. strain IAE139	*Arthrobacter sp.*	100%	0.0	98.02	1395	MK414833.1
Arthrobacter sp. strain L10	*Arthrobacter sp.*	100%	0.0	98.02	1371	MG646026.1
Glutamicibacter sp. strain L6	*Glutamicibacter sp.*	100%	0.0	98.02	1375	MG646025.1
Glutamicibacter halophytocola strain KLBMP 5180 (T)	*Glutamicibacter halophytocola*	100%	0.0	98.02	1448	MK424284.1
Glutamicibacter arilaitensis strain ebst40	*Glutamicibacter arilaitensis*	100%	0.0	98.02	1459	MG788347.1
Arthrobacter sp. djl-7H	*Arthrobacter sp. djl-7H*	100%	0.0	98.02	1372	KT230832.1
Arthrobacter sp. strain Art3	*Arthrobacter sp.*	100%	0.0	98.02	1372	MG708154.1
Glutamicibacter nicotianae strain AKPEW109	*Glutamicibacter nicotianae*	100%	0.0	98.02	1467	KX698104.1
Glutamicibacter nicotianae strain LCX26	*Glutamicibacter nicotianae*	100%	0.0	98.02	1387	KY646077.1
Glutamicibacter nicotianae strain LCX24	*Glutamicibacter nicotianae*	100%	0.0	98.02	1387	KY646076.1

>S17
GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCC
TTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACT
CTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAATACTTCCACTCGCATGG
GTGGGGGTTGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTGTTG
GTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTT
CGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAG
AAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTG
TCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTCTGTCGCGTCGGATGTGAAA
GCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGACTAGAGTGTGGTAGG
GGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGA
GCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGAACTAGGTGT
TGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGG
AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGC
GGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATCG
CCCGGAAAGCCGTAGAGATACGGCCCCCCTTGTGGTCGGGTGACAGGTGGTGCATG
GCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACTGCCG
GGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTG
GGCTGCACACGTGCTACAATGGCAGGTACAATGAGCTGCGATACCGCAAGGTGGAG
CGAATCTCAAAAAGCCTGTCTCAGTTCGGATTGGGGTCTGCAACTCGAATGAAGTCG
GAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACGTCACGAAAG

Hit (Genus):*Streptomyces*

Scientific name: *Streptomyces tauricus*

Query coverage: 100%

E value: 0.0

Percentage identity: 98.19&**Accession no.:** JX184904.1

The phylogenetic tree was constructed for *Streptomyces tauricus* YKIKM.MU02 with GenBank accession number MW785875.1 shown in **Figure 4.19** and the maximum likelihood species are listed in **Table 4.8.**

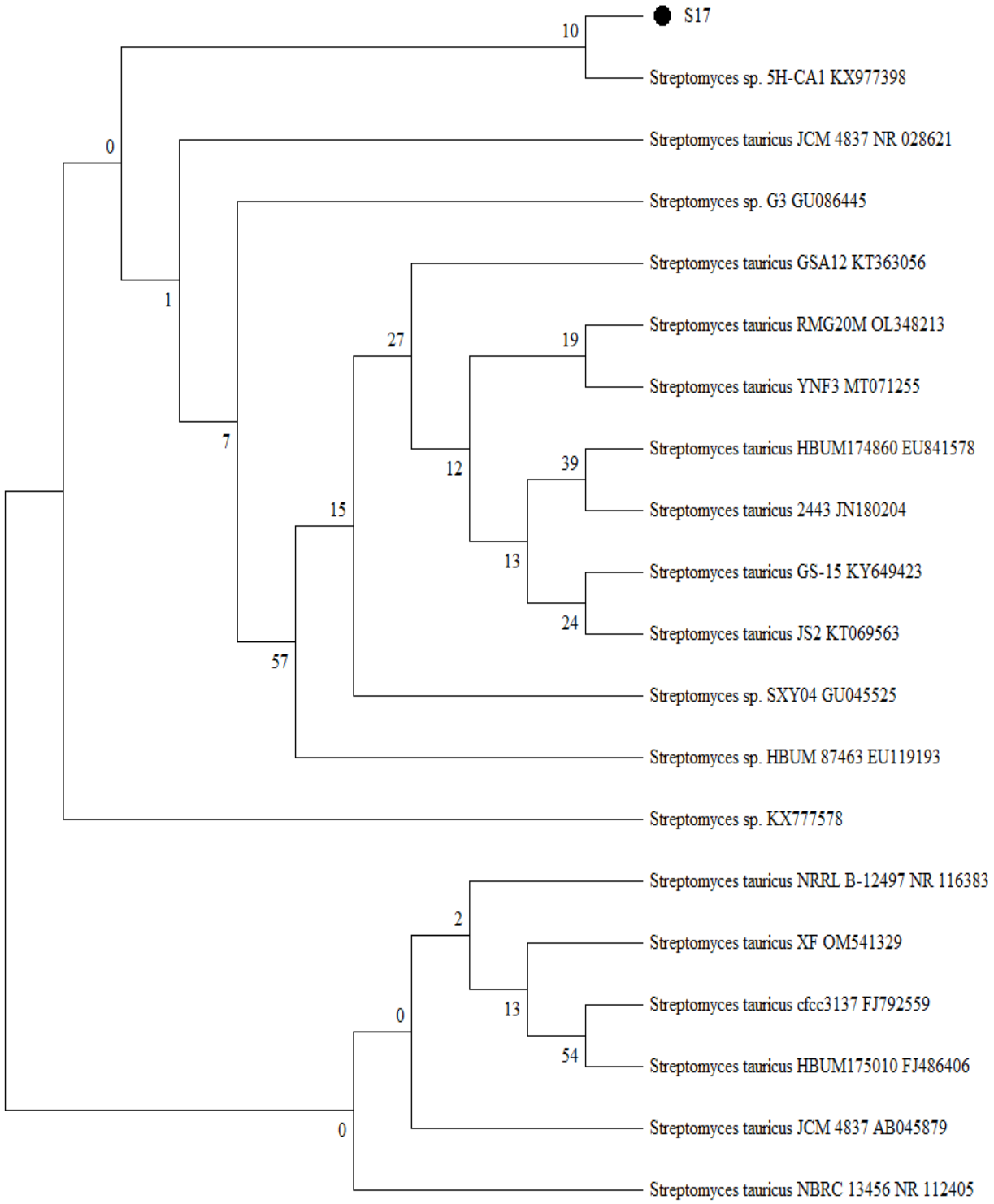

Figure 4.19 Phylogenetic tree of *Streptomyces tauricus*YKIKM.MU02 (S17) using Neighbor-Joining method.

Table 4.8Maximum relatedness species to *Streptomyces tauricus* YKIKM.MU02

Description	Scientific Name	Query Cover	E value	Per. Ident	Acc. Len	Accession
Streptomyces sp. strain 2H-HV12	*Streptomyces sp.*	100%	0.0	99.71	1512	KX777578.1
Streptomyces tauricus strain JCM 4837	*Streptomyces tauricus*	100%	0.0	99.71	1479	NR_028621.1
Streptomyces tauricus strain NRRL B-12497	*Streptomyces tauricus*	100%	0.0	99.71	1476	NR_116383.1
Streptomyces tauricus gene	*Streptomyces tauricus*	100%	0.0	99.71	1492	AB045879.1
Streptomyces sp. G3	*Streptomyces sp.* G3	99%	0.0	99.71	1492	GU086445.1
Streptomyces sp. strain 5H-CA1	*Streptomyces sp.*	100%	0.0	99.64	1520	KX977398.1
Streptomyces tauricus strain GSA12	*Streptomyces tauricus*	100%	0.0	99.64	1472	KT363056.1
Streptomyces sp. LRE541 chromosome,	*Streptomyces sp.* LRE541	100%	0.0	99.64	9966248	CP096907.1
Streptomyces tauricus strain RMG20M	*Streptomyces tauricus*	100%	0.0	99.64	1478	OL348213.1
Streptomyces sp. SXY04	*Streptomyces sp.* SXY04	100%	0.0	99.56	1526	GU045525.1
Streptomyces sp. HBUM87463	*Streptomyces sp.* HBUM 87463	100%	0.0	99.49	1524	EU119193.1
Streptomyces sp. 82165	*Streptomyces sp.* 82165	99%	0.0	99.64	1472	DQ008602.1
Streptomyces tauricus strain NBRC 13456	*Streptomyces tauricus*	99%	0.0	99.71	1468	NR_112405.1
Streptomyces sp. strain Md16	*Streptomyces sp.*	99%	0.0	99.49	1461	MN187450.1
Streptomyces sp. NEAU-GRX5	*Streptomyces sp. NEAU-GRX5*	100%	0.0	99.35	1521	KC202955.1
Streptomyces sp. NEAU-JM4	*Streptomyces sp.* NEAU-JM4	100%	0.0	99.27	1521	KM437944.1
Streptomyces sp. LJ-1	*Streptomyces sp.LJ-1*	100%	0.0	99.27	1520	KT122859.1
Streptomyces sp. NEAU-JM6	*Streptomyces sp.* NEAU-JM6	100%	0.0	99.27	1519	KM437942.1
Streptomyces sp. 81219	*Streptomyces sp.* 81219	98%	0.0	99.63	1466	AY996840.1

>S23
GCCTTTCTCTTTGGGATAAGCGCTGGAAACGGTGTCTAATACCAGATATTCATGCTTT
GCCGCATGGTGGGGTGTGGAAAGGCTGGAGGAAGATGGGCTCGCGGCCTATCAGCT
TGTTGGTGGGGTGATGGCCTACCAAGGCTTTGACGGGTAACCGGCCTGAGAGGGTG
ACCGGTCACACTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGGGGGATGACGG
CCTTCGGGTTGTAAACCTCTTTCGGTAAGGATGTAGGGCACTCTTTGGGGTGTTTGA
CTTTACTTGCTAAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGGCGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGCTCGTAGGCGGCGCGTCT
GTCGTGAAATCCAGTGGCTTAACTGCTGGTTTGCGGTGGGTACGGGCAGGCTTGAGT
GCGGTAGGGGAGATTGGAATTCTTGGTGTAGCGGTGAAATGCGCAGATATCAGGAA
GAACACCGGTGGCGAAGGCTGGTCTCTGGGCCGTTACTGACGCTGATGAGCGAAAG
CGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCA
CTGGGTGTGGGGGCCTTTGTGGTTTCTGTGCCGTAGCTAACGCGTTAAGTGCCCCGC
CTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACA
AGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTG
ACATGCGCTTTTGCTGCCAGAGATGGTGGTGGCCTTTTTGGCGGGGCGTGCAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTGTCTCGTGTTGCCAGCGCG

Hit (Genus): *Actinomyces*

Scientific name: *Boudabousia marimammalium*

Query coverage: 100%, **E value: 0.0**

Percentage identity: 98.90

Accession no.: MN022528.1

The *Actinomyces* isolate S23 was identified as *Boudabousia marimammalium* YKIKM.MU03 with GenBank accession numberMW898115.1and phylogenetic tree as shown in **Figure 4.20** and maximum likelihood species listed in **Table 4.9.** This culture was first reported from marine mammal i.e. dead seal and porpoise of UK by Lesley hoyles in 2001 (Hoyles et al., 2001). Our study thus demonstrated the occurrence of *Boudabousiamarimammalium* as a rare species from mangroves of Mangalore.

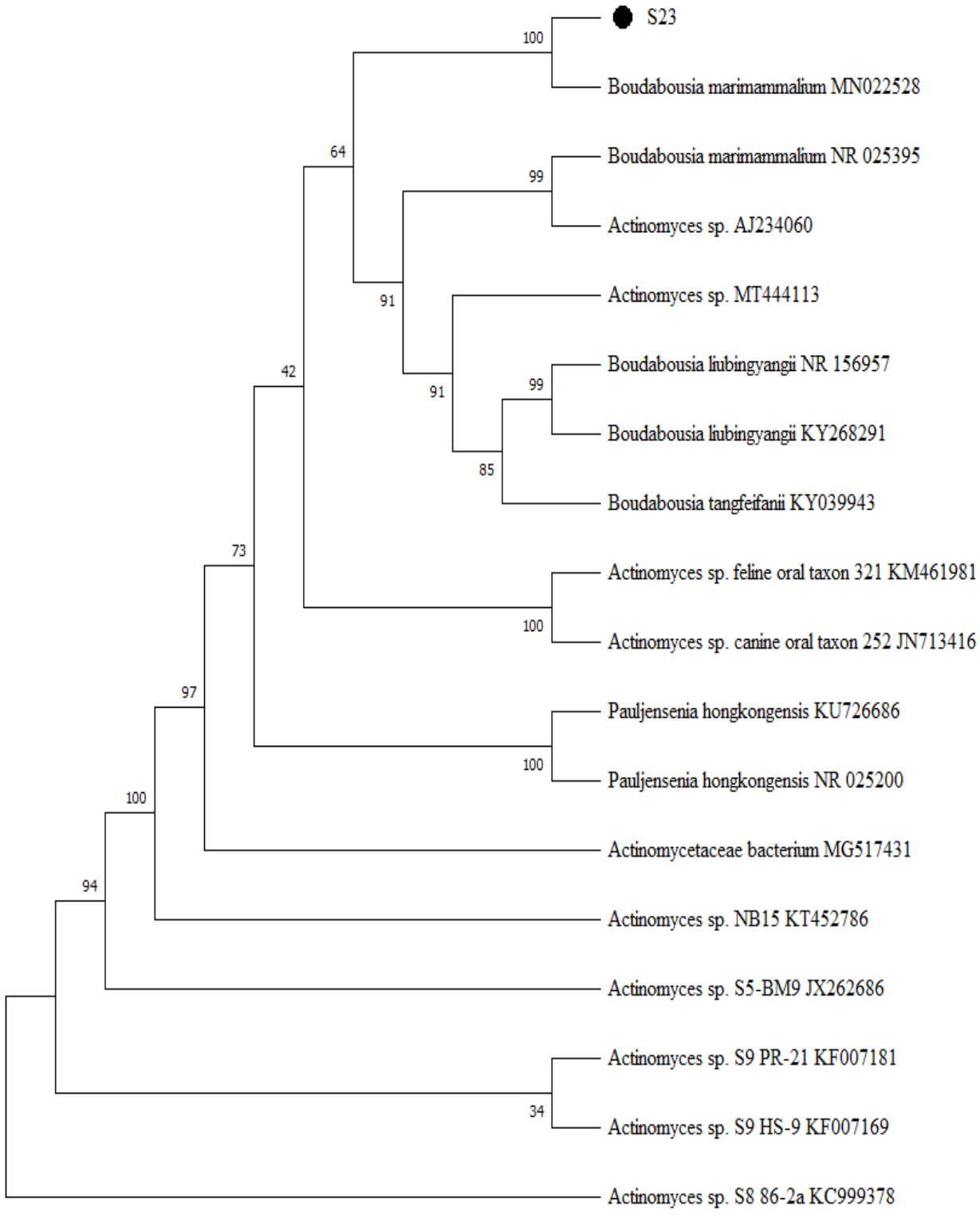

Figure 4.20 Phylogenetic tree of*Boudabousiamarimammalium*YKIKM.MU03 (S23) using Neighbor-Joining method.

Table 4.9Maximum relatedness species to *Boudabousia marimammalium*YKIKM.MU03

Description	Scientific Name	Query Cover	E value	Per. Ident	Acc. Len	Accession
Actinomyces marimammalium HBdwxiaoman	*Boudabousia marimammalium*	100%	0.0	98.90	1402	MN022528.1
Boudabousia marimammalium CCUG 41710	*Boudabousia marimammalium*	98%	0.0	93.02	1416	NR_025395.1
Actinomyces sp. M1349/94/1	*Actinomyces sp.*	98%	0.0	92.71	1378	AJ234060.1
Actinomyces sp. TAE3-ERU4	*Actinomyces sp.*	98%	0.0	92.21	1386	MT444113.1
Boudabousia liubingyangii VUL4_1	*Boudabousia liubingyangii*	98%	0.0	91.51	1455	NR_156957.1
Actinomyces sp. VUL4_3	*Boudabousia tangfeifanii*	98%	0.0	91.22	1457	KY039943.1
Boudabousia tangfeifanii VUL4_3	*Boudabousia tangfeifanii*	98%	0.0	91.22	2207832	CP017812.1
Actinomycesliubingyangii VUL4_2	*Boudabousialiubingyangii*	98%	0.0	91.10	1456	KY268291.1
Actinomyces sp. feline oral taxon 321 OH481	*Actinomyces sp.* feline oral	98%	0.0	90.55	1498	KM461981.1
Actinomycetaceae bacterium 9D3430KL	*Actinomycetaceae* bacterium	98%	0.0	90.44	1504	MG517431.1
Actinomyces sp. canine oral taxon 252 clone ZI340	*Actinomyces sp.*	98%	0.0	90.35	1498	JN713416.1
Pauljenseniahongkongensis DNF00842	*Pauljenseniahongkongensis*	98%	0.0	90.25	1090	KU726686.1
Pauljensenia hongkongensis HKU8	*Pauljensenia hongkongensis*	98%	0.0	90.25	1425	NR_025200.1
Actinomyces sp. S9 PR-21	*Actinomyces sp.* S9 PR-21	98%	0.0	90.15	1391	KF007181.1
Actinomyces sp. S9 HS-9	*Actinomyces sp.* S9 HS-9	98%	0.0	90.15	1364	KF007169.1
Actinomyces sp. S8 86-2a	*Actinomyces sp.* S8 86-2a	98%	0.0	90.15	1367	KC999378.1
Actinomyces sp. NB15	*Actinomyces sp.* NB15	98%	0.0	90.14	1406	KT452786.1

>S24
AAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGG
GATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGG
TACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGT
CGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAG
AGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG
GAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGA
AAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGG
GCACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGC
CCCGCCTGGGGAGTACGGCAA

Hit (Genus): *Streptomyces*

Scientific name: *Streptomyces viridiostaticus*

Query coverage: 100%

E value: 0.0, Percentage identity: 100%

Accession no.: MT672696.1

The isolates were documented in GenBank databases with accession number for S6 *Actinomyces* isolate MW647910.1 and named as *Glutamicibacter mysorens* strain YKIKM.MU. The S17 *Actinomyces* isolate provided with accession number MW785875.1 and named as *Streptomyces tauricus* strain YKIKM.MU02. Whereas S23 and S24 *Actinomyces* isolates were identified and received accession number MW898115.1 named as *Boudabousia marimammalium* YKIKM.MU03 and accession number MZ021336.1 named as *Streptomyces viridiostaticus*YKIKM.MU04. The *Streptomyces viridiostaticus* phylogenetic tree as shown in **Figure 4.21** and maximum likelihood species listed in **Table 4.10.**This present study reports the occurrences of these *Actinomyces* species in the mangrove habitat of Mangalore coastal region.

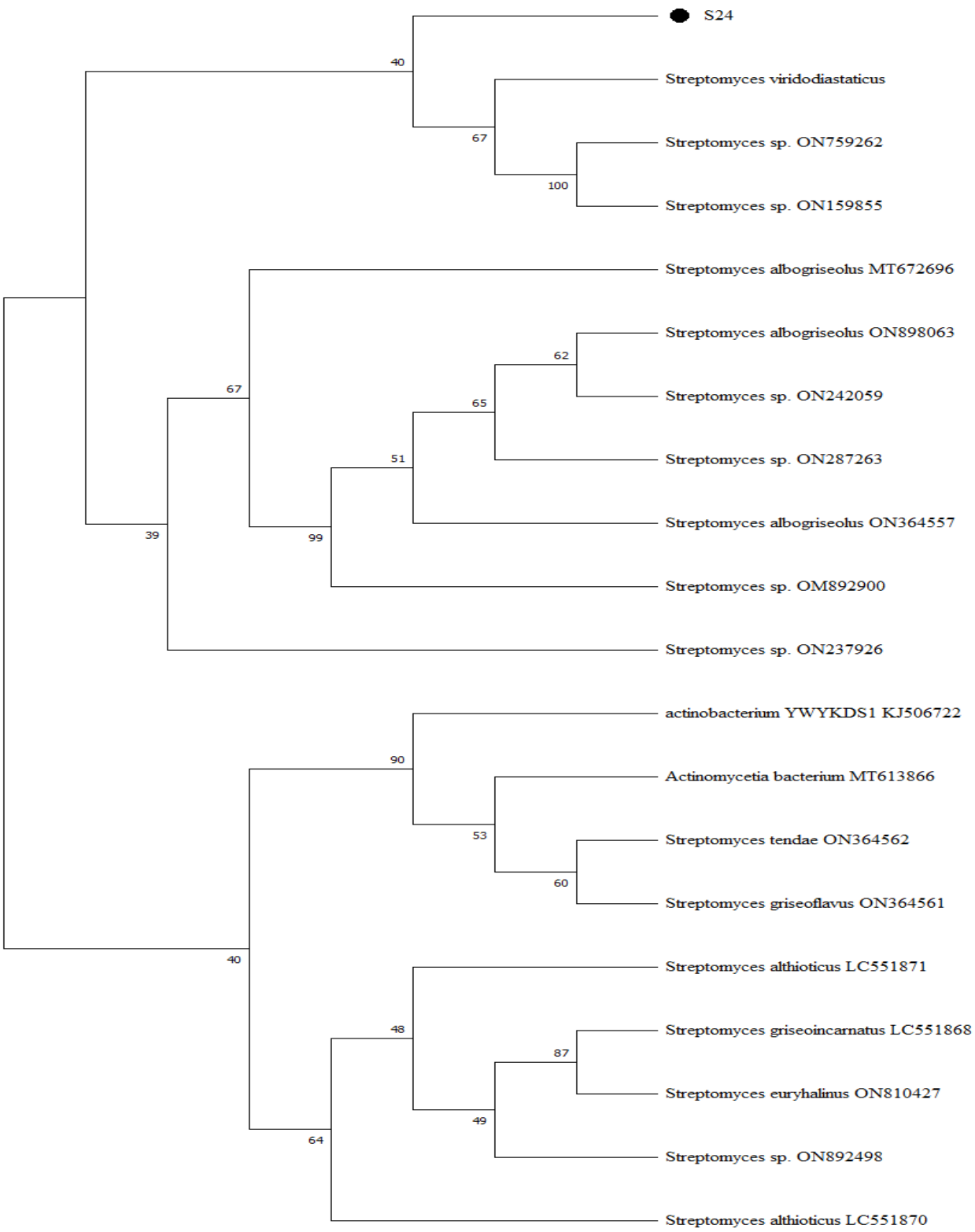

Figure 4.21 Phylogenetic tree of *Streptomyces viridiostaticus*YKIKM.MU04 (S24) using Neighbor-Joining method.

Table 4.10Maximum relatedness species to *Streptomyces viridiostaticus*YKIKM.MU04

Description	Scientific Name	Query Cover	E value	Per. Ident	Acc. Len	Accession
Streptomyces albogriseolus strain S19	*Streptomyces albogriseolus*	99%	0.0	100	791	MT672696.1
Actinobacterium YWYKDS1	*actinobacterium*	99%	0.0	100	1425	KJ506722.1
Actinomycetia bacterium strain XB-11-6-1	*Actinomycetia bacterium*	99%	0.0	100	1466	MT613866.1
Streptomyces althioticus P54-7	*Streptomyces althioticus*	99%	0.0	100	1328	LC551871.1
Streptomyces althioticus P54-15	*Streptomyces althioticus*	99%	0.0	100	1329	LC551870.1
Streptomyces griseoincarnatus P49-18	*Streptomyces griseoincarnatus*	99%	0.0	100	1303	LC551868.1
Streptomyces albogriseolus strain BL51	*Streptomyces albogriseolus*	99%	0.0	100	1403	ON898063.1
Streptomyces sp. strain ASENL1	*Streptomyces sp.*	99%	0.0	100	1461	ON892498.1
Streptomyces euryhalinus strain D119	*Streptomyces euryhalinus*	99%	0.0	100	1503	ON810427.1
Streptomyces sp. strain SNN211	*Streptomyces sp.*	99%	0.0	100	1405	ON759262.1
Streptomyces tendae strain 30K	*Streptomyces tendae*	99%	0.0	100	893	ON364562.1
Streptomyces griseoflavus strain 20K	*Streptomyces griseoflavus*	99%	0.0	100	896	ON364561.1
Streptomyces albogriseolus strain 8K	*Streptomyces albogriseolus*	99%	0.0	100	903	ON364557.1
Streptomyces sp. strain SD-20	*Streptomyces sp.*	99%	0.0	100	1162	OM892900.1
Streptomyces sp. strain Pv4-210	*Streptomyces sp.*	99%	0.0	100	1330	ON287263.1
Streptomyces sp. strain Pv4-49	*Streptomyces sp.*	99%	0.0	100	1340	ON242059.1
Streptomyces sp. strain Pv4-34	*Streptomyces sp.*	99%	0.0	100	1375	ON237926.1
Streptomyces sp. strain Msk05	*Streptomyces sp.*	99%	0.0	100	1400	ON159855.1
Streptomyces sp. BJ20 chromosome,	*Streptomyces sp.* BJ20	99%	0.0	100	7436964	CP094918.1
Streptomyces sp. strain SD-20	*Streptomyces sp.*	99%	0.0	100	1162	OM864302.1
Streptomyces griseorubens strain arb10-19garut	*Streptomyces griseorubens*	99%	0.0	100	1175	ON065869.1
Streptomyces variabilis strain BGPA1	*Streptomyces variabilis*	99%	0.0	100	1351	ON062185.1
Streptomyces sp. strain B148	*Streptomyces sp.*	99%	0.0	100	1154	ON041593.1
Streptomyces sp. strain B130	*Streptomyces sp.*	99%	0.0	100	1139	ON041575.1
Streptomyces sp. strain P11	*Streptomyces sp.*	99%	0.0	100	983	ON024738.1

The protein extracted from *Actinomyces* using simple and rapid method for extracting microbial protein according Bhaduri 1983 was subjected for further purification based on bioactivity guided purification procedures. After detecting and estimating the protein, *Actinomyces* peptides were subjected for size exclusion chromatographic separation using Sephadex-G10 column. The dimensions of the column setup of 1 X 110 cm (dxh) were equilibrated with two bed volumes of 0.05 M phosphate buffer pH 7.0. The flow rate of column was set to 10 ml/h for best separation. Bed size/volume is calculated using cylinder volume formula$\pi r^2 h$ where as π is 3.1428 and r^2 is 0.5 X 0.5 and height of the column was 110 cm about 86.427 ml for each bed volume.

A 20 mg of protein was loaded on top of the column and 2 ml fractions were collect and about 2.5 times (216.0675ml) bed volumes of protein elutions were collected. The absorbance of protein fractions was checked at 280nm and graph were plotted. The X-axis indicating fraction numbers and absorbance plotted on Y-axis for each fraction collected from Sephadex G-10 column chromatography.

The column elution profiles as shown in **Graph4.7-4.10** for bioactive protein separation from the *Glutamicibacter mysorens, Streptomyces tauricus, Boudabousia marimammalium* and *Streptomyces viridiostaticus* respectively. Antibacterial activity was analysed for peak fractions eluted from Sephadex G-10 column. A major peak at fraction number 99 of *Glutamicibacter mysorens* was estimated a yield of 9.38 % with fold purification of 10.66 % compared to crude protein extract as detailed in **Table 4.11** A peak at fraction number 115 of *Streptomyces tauricus* was estimated a yield of 8.077 percent with 11.923 folds purification compared to crude protein extract as detailed in **Table 4.12.** A peak at fraction number 87 of *Boudabousia marimammalium* was

estimated a yield of 8.48 percent with 11.52 fold purification compared to crude protein extract as detailed in **Table 4.13.** A peak at fraction number 72 of *Streptomyces viridiostaticus* was estimated a yield of 9.25 percent with 10.75 fold purification compared to crude protein extract as detailed in **Table 4.14**

Table 4.11 Purification chart of *Glutamicibacter mysorens* protein.

Sample	Protein mg/ml	Fold purification	% yield
Crude protein	2.0	1	100
Gel filtration (Sephadex G-10)	0.1875	10.66	9.38

Table 4.12 Purification chart of *Streptomyces tauricus* protein.

Sample	Protein mg/ml	Fold purification	% yield
Crude protein	2.6	1	100
Gel filtration (Sephadex G-10)	0.210	11.923	8.077

Table 4.13 Purification chart of *Boudabousia marimammalium* protein.

Sample	Protein mg/ml	Fold purification	% yield
Crude protein	2.3	1	100
Gel filtration (Sephadex G-10)	0.1950	11.52	8.48

Table 4.14 Purification chart of *Streptomyces viridiostaticus* protein.

Sample	Protein mg/ml	Fold purification	% yield
Crude protein	1.8	1	100
Gel filtration (Sephadex G-10)	0.1850	10.75	9.25

All the results obtained will be discussed in detail with relevant literature suitable illustrations and graphs in thesis.

Major findings and conclusion

The world ecological systems are classified in accordance with availability of nutrients by their distinguished flora and fauna to a specified region. The rich number of species diversity and their studies will offer for exploration and discovering of ubiquitously distributed species. Whereas the extreme stress habitats offers the isolation of rare species which produces discrete number of bioactive molecules for the treatment of various ailments.

The usage of inappropriate, regular and over dosage of antibiotics has increased microbial resistance in pathogenic microbes. The clinical exposure to MDR strains is a chronic and serious problem in present situation and is indicative of increased infectious diseases in near future. As the envoy of contagious disease, antibiotic resistance infectious agents have become source for unlimited human afflictions. Therefore, there is a need for search of alternate sources to have controlling effects on MDR strains and cellular diseases.

Beneficial microorganisms because of their adaptability to different environment and ease of culturing could offer a natural source for bioactive compounds to combat MDR and other drug resistant pathogens. Among the beneficial microorganisms, *Actinomyces* are prolific producers of bioactive compounds. One such environmental condition with unique habitat is the selection of mangroves, distinguished by high saline, temperature, tidal force are the stress factors affecting the adaptability of microbes.

Our study successfully isolated and explored 87 distinct *Actinomyces* isolates from the mangroves of Mangalore region with different morphological and microscopic features. Out of which four isolates were selected based on their prominent antimicrobial activity against broad spectrum pathogenic test cultures. These four isolates were identified through 16s rRNA gene

sequencing as *Glutamicibacter mysorens* with GenBank Acc. No. MW647910.1 *Streptomyces tauricus* with GenBank Acc. No. MW785875.1 *Boudabousia marimammalium* with GenBank Acc. No. MW898115.1 and *Streptomyces viridiostaticus* with GenBank Acc. No. MZ021336.1.The intracellular protein extracts from the selected *Actinomyces* showed effective antimicrobial activity against test pathogens. The efficient protein extraction method was followed for extracting intracellular protein from all the *Actinomyces* isolates. Antimicrobial activity was checked by well diffusion method and protein was detected by SDS-PAGE separation silver-nitrate staining. The bioactive peptide was separated after subjecting to Sephadex G-10 column chromatography and each peptide fraction checked for antimicrobial activity.

The bioactive peptides selected from all the four mangrove soil *Actinomyces* along in addition to antimicrobial activity also were analysed for anticancer effect on PC3 cell line. This resulted in the identification of microbial based bioactive peptides that have dual activity as antimicrobial and also as anticancer property. These microbial based bioactive peptides were characterized by LCMS/MS data record revealed the detection of bioactive peptides Kinetin-9-Riboside, Tryprostatin B, Surfactin C and Microcystin. Our study thus substantiates the microbial based bioactive peptides having both antimicrobial and anticancer activity with dual activity offer wide therapeutic applications to confer antimicrobial and anticancer in countering antibiotic resistance microbes and anticancer mechanisms targeting live cells.

Appendices; Publications:
From my thesis work

1. Karthik, Y., Kalyani M. I., 2022. Occurrence of *Streptomyces tauricus* in Mangrove soil of Mangalore region in Dakshina Kannada as a source for antimicrobial peptide; Journal of basic microbiology; Wiley publishers (Impact factor: 2.650).

2. **Karthik, Y.,** Kalyani, M.I., 2021 *Molecular Profiling of Glutamicibacter mysorens Strain YKIKM.MU and Bioactive Peptides Characterization for Antibacterial Activity. International journal of Pharmaceutical and Clinical Research. (ImpactFactor:1.668)*

3. Karthik, Y., Kalyani, M.I., Sheethal, K.S., Rakshitha, D., Bineesha, K.B., 2020. Cytotoxic and antimicrobial activities of microbial proteins from mangrove soil actinomycetes of Mangalore, Dakshina Kannada. Biomedicine. 40, 59-67 (ImpactFactor:0.1 Elsevier Indian CitationIndex)

Outside the Thesis work

4. Gowthami G. A., **Yalpi Karthik**., Das, Subhankar., and Kalyani, M.I., 2021 Study of antibacterial, anti-proliferative and pro- apoptotic potential of the cell extracts of endophytic fungi and bacteria isolated from *Pajanelialongifolia*. (ImpactFactor:0.693)

5. Ganesh Prasad D.N., **Yalpi Karthik**, Sachin H.R. and A.H. Sneharani 2021. Screening for extracellular enzyme activities by bacteria isolated from honeybee, *Apis florea* gut Biomedicine. 41, (Impact Factor:0.1 Elsevier Indian Citation Index)

6. Srinivasa Krishnappa, **Yalpi Karthik**, Manjula Shantaram. Assessment of Different Urinary Protein Precipitation Methods for Protein profiling by Mass Spectrometry. 2021 Asian Journal of Pharmaceutics (ImpactFactor:0.987)

7. Avinash Irappa Ammanagi1, Shivasharana C.T, Krishnaveni R, Abhijeeth Shivappa Badiger, Vijaya Kumar Ramaraj, Srinath B S and **Yalpi Karthik**; 2021; A Biotechnological Approach to Optimization and Production of Melanin by *Brevibacillus invocatus* strain IBA, under submerged fermentation Biomedicine (Impact Factor: 0.1 Elsevier Indian Citation Index)

Presentations:

1. An Oral Presentation was done on "**Production and characterization of biologically important molecules from *Streptomyces tauricus* in Mangrove soil of Mangalore region"** at 2 days National Conference on "Integrated Microbial Technology for Sustainable Future" (IMTSF-2022) on 7th and 8th of July 2022 Organised by Department of Microbiology, Mangalore University, Jnana Kaveri, Chikka Aluvara,Kodagu-571232.

2. An Oral Presentation was done on "**Molecular Profiling of *Glutamicibacter mysorens* Strain YKIKM.MU and Bioactive Peptides Characterization for Antibacterial Activity**" at International Conference was organised by Indian Association of Biomedical Scientists, Chennai.

3. An poster presentation was done on **"Qualitative identification of bioactive compounds from unicellular filamentous bacteria"** International Workshop was organized by Joint Workshop on Biology and Applications of Actinomycetes at Department of Studiesin Microbiology, University of Mysore, Manasagangotri, Mysore, India570006

4. An Oral Presentation on **"Bioactive Peptides from Mangrove Actinomycetes and Characterization for Antimicrobial Activity" at** National Conference on "Secondary Metabolites & Health care" held in Alva's College, Moodbidre, D.K. Karnataka on 23RDFebruary2019.

Organizing Member:

1. Organizing member for 2 days National Conference on "Integrated Microbial Technology for Sustainable Future"-(IMTSF-2022) Organised by Department of Microbiology, Mangalore University, Jnana Kaveri, Chikka Aluvara,Kodagu-571232.

2. Organizing member for 3 days online Workshop on Advancement in Applied Microbiology and Biotechnology 2021 organized by Department of Microbiology, Mangalore University, Jnana Kaveri, Chikka Aluvara,Kodagu-571232.

3. Organizing member for 39th IABMS international conference (Current Concepts on the role of Indian medicine and phytoceuticals in maintenance of health) held in Department of Biochemistry, Jnana

Kaveri Campus, Mangalore University from 15- 17th Nov. 2018

Participated in International Conferences:

1. Participated in virtual Conference on "World Earth Day Restore Our Earth" held on 22nd April 2021 organized by Surana Educational Institutions in association with Karnataka Science and Technology Academy, Bangalore.

2. Participated in virtual International Conference on "Experiments in gender main streaming India and Canada held on 16th to 17th March 2021 organized by UGC centre for women Sciences Mangalore University,Mangalagangotri-574199

3. Participated in 39th IABMS international Conference (Current Concepts on the role of Indian medicine and phytoceuticals in maintenance of health) held in Dept. of Biochemistry, Jnana Kaveri Campus, Mangalore University from 15- 17th Nov. 2018.

4. Participated in International Conference on Biochemical Investigations: A Corner stone to Clinical Care" on 27th July 2013 held in Kasturba Medical College (Manipal),Mangalore.

5. Participated in International Conference on Radiation Biology & Clinical Applications held in K.S. Hegde Medical Academy (NITTE) on 25-27th October2013.

Memberships:

1. Life Member of Institute of Scholars (InSc a unit of SDPL) from Karnataka Zone.

2. Founder & Life Member of Indian Society for Vector Ecology (SOVE)-2018for Indian Region.

3. Member of the editorial committee for the Jnana Kaveri News Bulletin of Jnana Kaveri Campus, Mangalore University.

Workshops attended:

Sl. No.	Year	Name of Course	Duration	Organized by
1.	2021	National Level 5 Days Faculty Development Program on "Material Characterization Techniques"	5 days 2nd to 6th March 2021	Bhavan's Vivekananda College of Science, Humanities & Commerce Sainikpuri, Hyderabad-560094
2.	2021	Online Faculty Development Program on Nanomaterials: Experimental Design and Theoretical Knowledge	8 Days13th to 20th Feb 2021	E & ICT Academy, National Institute of Technology, Warangal in association with Indian Institute of Information Technology Design and Manufacturing, Kurnool.
3.	2020	Hands on workshopon Metagenomic Data Analysis	5 Days 10th to14th Feb 2020	Jointly Organised by VNSGU and Gujurat Biotechnology Research Centre, Gandhinagar, Gujurat
4.	2019	Joint Workshop on Biology and Applications of Actinomycetes	2 Days 31st Oct to1st Nov 2019	Department of Studies in Microbiology University of Mysore, Manasagangotri, Mysore,India 570006
5.	2019	"Plant Microbial Interaction for the production of therapeutic Compounds"	10 Days 17th to 26th Oct 2019	Dept. of P. G. Studies & Research in APPLIED BOTANY, Jnana Sahyadri, Shankaraghatta-577451 Shimoga Dist., Karnataka, INDIA
6.	2019	Hands on training Six Day Hands on workshop on "Bioinformatics &Biostatistics"	6 Days 24th to 29thJune 2019	School of Science RK University, Kasturbadham, Rajkot -360020
7.	2019	Hands on Training Workshop on "Basic Cell Culture Technology"	4 Days 6th to 9th May 2019	National Centre for Cell Science, Cell Repository, Pune
8.	2018	Pre-conference Workshop on "Basics of Cell Culture and applications indrug discovery"	1day	Dept. of Studies and Research in Biochemistry, Jnana Kaveri, Mangalore University

References

Abdillah, S., Tambunan, R.M., Farida, Y., Sandhiutami, N.M.D., Dewi, R.M., 2015. Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia. Asian Pacific Journal of Tropical Disease 5, 454–457.

Ahad, W., 2020. Clinical Management of Bovine Actinomycosis (Lumpy Jaw) in Cattle: A Case Report 10, 24868–24870.

Alapati, K., Muvva, V., 2013. Evaluation of bioactive compounds produced by Nocardia levis MK-VL_113 & Streptomyces tendae TK-VL_333 for cytotoxic activity. Indian Journal of Medical Research 137, 391–393.

Auer, S., Wojna, A., Hell, M., 2009. ESBL Producing Escherichia coli in ambulatory Urinary Tract Infections -Oral Treatment Options?

Balasubramanian, B., Benit, N., Agastian, P., Almaary, K.S., Dawoud, T.M., Elbadawi, Y.B., Mubarak, A., Alfadul, M.S., Aljowaie, R.M., 2021. Carbapenemases producing Klebsiella pneumoniae from the pus of hospitalized patients: In-vitro antibiotic properties of Streptomyces against multidrug resistant infectious bacteria. Journal of Infection and Public Health 14, 892–897. https://doi.org/10.1016/j.jiph.2021.05.006

Binnie, C., Cossar, J.D., Stewart, D.I.H., 1997. Heterologous biopharmaceutical protein expression in Streptomyces. Trends in Biotechnology 15, 315–320. https://doi.org/10.1016/S0167-7799(97)01062-7

Bjornsti, M.-A., Knab, A.M., Benedetti, P., 1994. YeastSaccharomyces cerevisiae as a model system to study the cytotoxic activity of the antitumor drug camptothecin. Cancer chemotherapy and pharmacology 34, S1–S5.

Charousová, I., Medo, J., Hleba, L., Císarová, M., Javoreková, S., 2019. Antimicrobial activity of actinomycetes and characterization of actinomycin-producing strain KRG-1 isolated from Karoo, South Africa. Braz. J. Pharm. Sci. 55, e17249. https://doi.org/10.1590/s2175-97902019000217249

Chaudhary, Hotam Singh, Soni, B., Shrivastava, A.R., Shrivastava, S., 2013. Diversity and Versatility of Actinomycetes and its Role in Antibiotic Production. Journal of Applied Pharmaceutical Science 3, 12. https://doi.org/10.7324/JAPS.2013.38.S14

Chaudhary, Hotam S., Yadav, J., Shrivastava, A.R., Singh, S., Singh, A.K., Gopalan, N., 2013. Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). J Adv Pharm Technol Res 4, 118–123. https://doi.org/10.4103/2231-4040.111528

Claesen, J., Bibb, M.J., 2011. Biosynthesis and Regulation of Grisemycin, a New Member of the Linaridin Family of Ribosomally Synthesized Peptides Produced by Streptomyces griseus IFO 13350. Journal of Bacteriology. https://doi.org/10.1128/JB.00171-11

Clark Mason, J., Richards, M., Zimmermann, W., Broda, P., 1988. Identification of extracellular proteins from actinomycetes responsible for the solubilisation of lignocellulose. Appl Microbiol Biotechnol 28, 276–280. https://doi.org/10.1007/BF00250455

Collin, F., Maxwell, A., 2019. The Microbial Toxin Microcin B17: Prospects for the Development of New Antibacterial Agents. J Mol Biol 431, 3400–3426. https://doi.org/10.1016/j.jmb.2019.05.050

Corbaz, R., Gregory, P.H., Lacey, M.E.Y., 1963. Thermopilic and Mesophilic Actinomycetes in Mouldy Hay. Microbiology 32, 449–455. https://doi.org/10.1099/00221287-32-3-449

Dardić, D., Lauro, G., Bifulco, G., Laboudie, P., Sakhaii, P., Bauer, A., Vilcinskas, A., Hammann, P.E., Plaza, A., 2017. Svetamycins A–G, Unusual Piperazic Acid-Containing Peptides from Streptomyces sp. J. Org. Chem. 82, 6032–6043. https://doi.org/10.1021/acs.joc.7b00228

Desouky, S.E., Shojima, A., Singh, R.P., Matsufuji, T., Igarashi, Y., Suzuki, T., Yamagaki, T., Okubo, K., Ohtani, K., Sonomoto, K., Nakayama, J., 2015. Cyclodepsipeptides produced by actinomycetes inhibit cyclic-peptide-mediated quorum sensing in Gram-positive bacteria. FEMS Microbiology Letters 362, fnv109. https://doi.org/10.1093/femsle/fnv109

Donato, D.C., Kauffman, J.B., Mackenzie, R.A., Ainsworth, A., Pfleeger, A.Z., 2012. Whole-island carbon stocks in the tropical Pacific: Implications for mangrove conservation and upland restoration. Journal of Environmental Management 97, 89–96. https://doi.org/10.1016/j.jenvman.2011.12.004

Donato, D.C., Kauffman, J.B., Murdiyarso, D., Kurnianto, S., Stidham, M., Kanninen, M., 2011. Mangroves among the most carbon-rich forests in the tropics. Nature Geosci 4, 293–297. https://doi.org/10.1038/ngeo1123

Doshi, D.V., Maniyar, J.P., Bhuyan, S.S., Mujumdar, S.S., 2010. Studies on bioemulsifier production by Actinopolyspora sp. A18 isolated from garden soil. INDIAN J BIOTECHNOL 6.

Duke, N.C., Nagelkerken, I., Agardy, T., Wells, S., Lavieren, H. van, Bochove, J.-W. van, Sullivan, E., Nakamura, T., UNEP World Conservation Monitoring Centre, United Nations Environment Programme, 2014. The importance of mangroves to people: a call to action.

Duraiappah, A.K., Naeem, S., Agardy, T., Ash, N.J., Cooper, H.D., Diaz, S., Faith, D.P., Mace, G., McNeely, J.A., Mooney, H.A., Oteng-Yeboah, A.A., Pereira, H.M., Polasky, S., Prip, C., Reid, W.V., Samper, C., Schei, P.J., Scholes, R., Schutyser, F., Van Jaarsveld, A., 2005. Ecosystems and human well-being: biodiversity synthesis; a report of the Millennium Ecosystem Assessment. World Resources Institute.

El Karkouri, A., Assou, S.A., El Hassouni, M., 2019. Isolation and screening of actinomycetes producing antimicrobial substances from an extreme Moroccan biotope. Pan Afr Med J 33, 329. https://doi.org/10.11604/pamj.2019.33.329.19018

Elayaraja, S., Annamalai, N., Mayavu, P., Balasubramanian, T., 2014. Production, purification and characterization of bacteriocin from Lactobacillus murinus AU06 and its broad antibacterial spectrum. Asian Pac J Trop Biomed 4, S305-311. https://doi.org/10.12980/APJTB.4.2014C537

Ganesan, P., Reegan, A.D., David, R.H.A., Gandhi, M.R., Paulraj, M.G., Al-Dhabi, N.A., Ignacimuthu, S., 2017. Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India. Alexandria Journal of Medicine 53, 101–110. https://doi.org/10.1016/j.ajme.2016.03.004

Gilman, R.H., Hornick, R.B., 1976. Duodenal isolation of Salmonella typhi by string capsule in acute typhoid fever. Journal of Clinical Microbiology 3, 456–457.

Goldwater, P.N., Bettelheim, K.A., 2012. Treatment of enterohemorrhagic Escherichia coli (EHEC) infection and hemolytic uremic syndrome (HUS). BMC medicine 10, 1–8.

Goodfellow, M., Stach, J.E.M., Brown, R., Bonda, A.N.V., Jones, A.L., Mexson, J., Fiedler, H.-P., Zucchi, T.D., Bull, A.T., 2012. Verrucosispora maris sp. nov., a novel deep-sea actinomycete isolated from a marine sediment which produces abyssomicins. Antonie van Leeuwenhoek 101, 185–193. https://doi.org/10.1007/s10482-011-9651-5

Gould, D., 2010. Causes, prevention and treatment of Escherichia coli infections. Nursing Standard (through 2013) 24, 50.

Gunatilaka, A.A.L., 2006. Natural Products from Plant-Associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. J. Nat. Prod. 69, 509–526. https://doi.org/10.1021/np058128n

Hamaki, T., Suzuki, M., Fudou, R., Jojima, Y., Kajiura, T., Tabuchi, A., Sen, K., Shibai, H., 2005. Isolation of novel bacteria and actinomycetes using soil-extract agar medium. Journal of Bioscience and Bioengineering 99, 485–492. https://doi.org/10.1263/jbb.99.485

Higa, D.T., n.d. BENEFICIAL AND EFFECTIVE MICROORGANISMS for a SUSTAINABLE AGRICULTURE AND ENVIRONMENT 16.

Hoyles, L., Pascual, C., Falsen, E., Foster, G., Grainger, J.M., Collins, M.D., 2001. Actinomyces marimammalium sp. nov., from marine mammals. International journal of systematic and evolutionary microbiology 51, 151–156.

Hyi-Seung Lee, †, Hee Jae Shin, †, Kyoung Hwa Jang, ‡, Tae Sik Kim, †, Ki-Bong Oh, § and, Jongheon Shin*, ‡, 2005. Cyclic Peptides of the Nocardamine Class from a Marine-Derived Bacterium of the Genus Streptomyces [WWW Document]. ACS Publications. https://doi.org/10.1021/np040220g

Igarashi, M., Sawa, R., Kinoshita, N., Hashizume, H., Nakagawa, N., Homma, Y., Nishimura, Y., Akamatsu, Y., 2008. Pargamicin A, a Novel Cyclic Peptide Antibiotic from Amycolatopsis sp. J Antibiot 61, 387–393. https://doi.org/10.1038/ja.2008.54

Ismail, S., Jiang, B., Nasimi, Z., Inam-Ul-Haq, M., Yamamoto, N., Danso Ofori, A., Khan, N., Arshad, M., Abbas, K., Zheng, A., 2020. Investigation of Streptomyces scabies Causing Potato Scab by Various Detection Techniques, Its Pathogenicity and Determination of Host-Disease Resistance in Potato Germplasm. Pathogens 9, E760. https://doi.org/10.3390/pathogens9090760

Janiszewska, J., Sowińska, M., Rajnisz, A., Solecka, J., \Lącka, I., Milewski, S., Urbańczyk-Lipkowska, Z., 2012. Novel dendrimeric lipopeptides with antifungal activity. Bioorganic & medicinal chemistry letters 22, 1388–1393.

Johansson E, Claesson R, Van Dijken J W V, 2009. Antibacterial effect of ozone on cariogenic bacterial species. Journal of Dentistry 37, 449–453. https://doi.org/10.1016/j.jdent.2009.02.004

Kalyani, M.I., 2017. Peptide Analysis from Soil Actinomycetes Exhibiting Antimicrobial and Antiproliferative Activities. 1 8. https://doi.org/10.22377/ijpba.v8i06.1555

Kirby, R., Sangal, V., Tucker, N.P., Zakrzewska-Czerwińska, J., Wierzbicka, K., Herron, P.R., Chu, C.-J., Chandra, G., Fahal, A.H., Goodfellow, M., Hoskisson, P.A., 2012. Draft Genome Sequence of the Human Pathogen Streptomyces somaliensis, a Significant Cause of Actinomycetoma. Journal of Bacteriology 194, 3544–3545. https://doi.org/10.1128/JB.00534-12

Kitani, S., Ikeda, H., Sakamoto, T., Noguchi, S., Nihira, T., 2009. Characterization of a regulatory gene, aveR, for the biosynthesis of avermectin in Streptomyces avermitilis. Applied microbiology and biotechnology 82, 1089–96. https://doi.org/10.1007/s00253-008-1850-2

Kotiranta, A., Lounatmaa, K., Haapasalo, M., 2000. Epidemiology and pathogenesis of Bacillus cereus infections. Microbes and Infection 2, 189–198. https://doi.org/10.1016/S1286-4579(00)00269-0

Krishanti, N., 2018. Antimicrobial Production by an Actinomycetes Isolated from the Termite Nest 8, 279–288. https://doi.org/10.11594/jtls.08.03.10

Kuhad, R., 2012. Microbes and their Role in Sustainable Development. Indian journal of microbiology 52, 309–313. https://doi.org/10.1007/s12088-012-0267-x

Lammertyn, E., Anné, J., 1998. Modifications of Streptomyces signal peptides and their effects on protein production and secretion. FEMS Microbiology Letters 160, 1–10. https://doi.org/10.1111/j.1574-6968.1998.tb12882.x

Lederman, E.R., Crum, N.F., 2005. Pyogenic Liver Abscess with a Focus on Klebsiella pneumoniae as a Primary Pathogen: An Emerging Disease with Unique Clinical Characteristics. Official journal of the American College of Gastroenterology | ACG 100, 322–331.

Lowy, F.D., 1998. *Staphylococcus aureus* Infections. N Engl J Med 339, 520–532. https://doi.org/10.1056/NEJM199808203390806

Mahmoudi, F., Baradaran, B., Dehnad, A., Shanehbandi, D., Mohamed Khosroshahi, L., Aghapour, M., 2016. The immunomodulatory activity of secondary metabolites isolated from Streptomyces calvus on human peripheral blood mononuclear cells. Br J Biomed Sci 73, 97–103. https://doi.org/10.1080/09674845.2016.1188476

Mevaere, J., Goulard, C., Schneider, O., Sekurova, O.N., Ma, H., Zirah, S., Afonso, C., Rebuffat, S., Zotchev, S.B., Li, Y., 2018. An orthogonal system for heterologous expression of actinobacterial lasso peptides in Streptomyces hosts. Sci Rep 8, 1–11. https://doi.org/10.1038/s41598-018-26620-0

Moffatt, C.R.M., Musto, J., Pingault, N., Miller, M., Stafford, R., Gregory, J., Polkinghorne, B.G., Kirk, M.D., 2016. Salmonella Typhimurium and Outbreaks of Egg-Associated Disease in Australia, 2001 to 2011. Foodborne Pathog Dis 13, 379–385. https://doi.org/10.1089/fpd.2015.2110

Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., Donadio, S., 2014. Discovering new bioactive molecules from microbial sources. Microb Biotechnol 7, 209–220. https://doi.org/10.1111/1751-7915.12123

Murphy, T.F., Brauer, A.L., Eschberger, K., Lobbins, P., Grove, L., Cai, X., Sethi, S., 2008. Pseudomonas aeruginosa in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 177, 853–860. https://doi.org/10.1164/rccm.200709-1413OC

Myla, J., Chandrasekaran, R., Muthu, R.S., 2016. Studies on Diversity of Actinomycetes in Oil Contaminated Soil. Biosciences Biotechnology Research Asia 7, 231–238. https://doi.org/10.13005/bbra

Neter, E.R., Farrar, R.H., 1943. Proteus vulgaris and proteus morganii in diarrheal disease of infants. Am. J. Dig. Dis. 10, 344–347. https://doi.org/10.1007/BF03001475

Nikolaitchouk, N., Hoyles, L., Falsen, E., Grainger, J.M., Collins, M.D., 2000. Characterization of Actinomyces isolates from samples from the human urogenital tract: description of Actinomyces urogenitalis sp. nov. International Journal of Systematic and Evolutionary Microbiology 50, 1649–1654. https://doi.org/10.1099/00207713-50-4-1649

Pesic, A., Baumann, H.I., Kleinschmidt, K., Ensle, P., Wiese, J., Süssmuth, R.D., Imhoff, J.F., 2013. Champacyclin, a New Cyclic Octapeptide from Streptomyces Strain C42 Isolated from the Baltic Sea. Marine Drugs 11, 4834–4857. https://doi.org/10.3390/md11124834

Poffe, M., Srinivasan, M.R., Venkatesan, B., 2014. Crude protein extract of Actinobacteria exhibits antibacterial activity against Salmonella typhi. Current Microbiology 3, 319–326.

Prakasham, R.S., Kumar, B.S., Kumar, Y.S., Kumar, K.P., 2014. Production and Characterization of Protein Encapsulated Silver Nanoparticles by Marine Isolate

Streptomyces parvulus SSNP11. Indian J Microbiol 54, 329–336. https://doi.org/10.1007/s12088-014-0452-1

Promnuan, Y., Kudo, T., Chantawannakul, P., 2009. Actinomycetes isolated from beehives in Thailand. World J Microbiol Biotechnol 25, 1685–1689. https://doi.org/10.1007/s11274-009-0051-1

Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., Moënne-Loccoz, Y., 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321, 341–361. https://doi.org/10.1007/s11104-008-9568-6

Rajeswari, P., Jose, P.A., Amiya, R., Jebakumar, S.R.D., 2015. Characterization of saltern based Streptomyces sp. and statistical media optimization for its improved antibacterial activity. Front Microbiol 5, 753. https://doi.org/10.3389/fmicb.2014.00753

Ramachandran, R., Kuppusamy, A., 2016. In vitro antimicrobial activity of marine Actinobacteria isolates from Pulicat Lake, Tamil Nadu, India. Asian Journal of Pharmaceutical and Clinical Research 9.

Renner, M.K., Shen, Y.-C., Cheng, X.-C., Jensen, P.R., Frankmoelle, W., Kauffman, C.A., Fenical, W., Lobkovsky, E., Clardy, J., 1999. Cyclomarins A−C, New Antiinflammatory Cyclic Peptides Produced by a Marine Bacterium (Streptomyces sp.). J. Am. Chem. Soc. 121, 11273–11276. https://doi.org/10.1021/ja992482o

Román-Hurtado, F., Sánchez-Hidalgo, M., Martín, J., Ortiz-López, F.J., Carretero-Molina, D., Reyes, F., Genilloud, O., 2020. One pathway, two cyclic pentapeptides: heterologous expression of BE-18257 A-C and pentaminomycins A-E from Streptomyces cacaoi CA-170360. https://doi.org/10.1101/2020.10.23.352575

Sapkota, A., Thapa, A., Budhathoki, A., Sainju, M., Shrestha, P., Aryal, S., 2020. Isolation, Characterization, and Screening of Antimicrobial-Producing Actinomycetes from Soil Samples. International Journal of Microbiology 2020.

Sawyer, E.B., Claessen, D., Haas, M., Hurgobin, B., Gras, S.L., 2011. The Assembly of Individual Chaplin Peptides from Streptomyces coelicolor into Functional Amyloid Fibrils. PLOS ONE 6, e18839. https://doi.org/10.1371/journal.pone.0018839

Shrestha, B., Nath, D.K., Maharjan, A., Poudel, A., Pradhan, R.N., Aryal, S., 2021. Isolation and Characterization of Potential Antibiotic-Producing Actinomycetes from Water and Soil Sediments of Different Regions of Nepal. International Journal of Microbiology 2021, e5586165. https://doi.org/10.1155/2021/5586165

Sohda, K., Hiramoto, M., Suzumura, K., Takebayashi, Y., Suzuki, K., Tanaka, A., 2005. YM-216391, a Novel Cytotoxic Cyclic Peptide from Streptomyces nobilis. J Antibiot 58, 32–36. https://doi.org/10.1038/ja.2005.3

Solecka, J., Zajko, J., Postek, M., Rajnisz, A., 2012. Biologically active secondary metabolites from Actinomycetes. Open Life Sciences 7, 373–390.

Tian, S., Yang, Y., Liu, K., Xiong, Z., Xu, L., Zhao, L., 2014. Antimicrobial metabolites from a novel halophilic actinomycete Nocardiopsis terrae YIM 90022. Natural Product Research 28, 344–346. https://doi.org/10.1080/14786419.2013.858341

Tiwari, K., Gupta, R.K., 2012. Rare actinomycetes: a potential storehouse for novel antibiotics. Critical Reviews in Biotechnology 32, 108–132. https://doi.org/10.3109/07388551.2011.562482

Tiwari, K., Upadhyay, D.J., Mösker, E., Süssmuth, R., Gupta, R.K., 2015. Culturable bioactive actinomycetes from the Great Indian Thar Desert. Ann Microbiol 65, 1901–1914. https://doi.org/10.1007/s13213-014-1028-3

Um, S., Choi, T.J., Kim, H., Kim, B.Y., Kim, S.-H., Lee, S.K., Oh, K.-B., Shin, J., Oh, D.-C., 2013. Ohmyungsamycins A and B: Cytotoxic and Antimicrobial Cyclic Peptides Produced by Streptomyces sp. from a Volcanic Island. J. Org. Chem. 78, 12321–12329. https://doi.org/10.1021/jo401974g

Vasilchenko, A.S., Julian, W.T., Lapchinskaya, O.A., Katrukha, G.S., Sadykova, V.S., Rogozhin, E.A., 2020. A Novel Peptide Antibiotic Produced by Streptomyces roseoflavus Strain INA-Ac-5812 With Directed Activity Against Gram-Positive Bacteria. Frontiers in Microbiology 11.

Williams, S.T., Davies, F.L.Y. 1965, n.d. Use of Antibiotics for Selective Isolation and Enumeration of Actinomycetes in Soil. Microbiology 38, 251–261. https://doi.org/10.1099/00221287-38-2-251

Xu, D.-B., Ye, W.-W., Han, Y., Deng, Z.-X., Hong, K., 2014. Natural Products from Mangrove Actinomycetes. Marine Drugs 12, 2590–2613. https://doi.org/10.3390/md12052590

Yin, P., Wang, Y.-H., Zhang, S.-L., Chu, J., Zhuang, Y.-P., Wang, M.-L., Zhou, J., 2008. Isolation of soluble proteins from an industrial strain Streptomyces avermitilis in complex culture medium for two-dimensional gel electrophoresis. J Microbiol Methods 73, 105–110. https://doi.org/10.1016/j.mimet.2008.02.012

Zong, C., Cheung-Lee, W.L., Elashal, H.E., Raj, M., Link, A.J., 2018. Albusnodin: an acetylated lasso peptide from Streptomyces albus. Chem. Commun. 54, 1339–1342. https://doi.org/10.1039/C7CC08620B

Zucchi, T.D., Bonda, A.N.V., Frank, S., Kim, B.-Y., Kshetrimayum, J.D., Goodfellow, M., 2012. Amycolatopsis bartoniae sp. nov. and Amycolatopsis bullii sp. nov., mesophilic actinomycetes isolated from arid Australian soils. Antonie van Leeuwenhoek 102, 91–98. https://doi.org/10.1007/s10482-012-9716-0